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UTILITY PATENT APPLICATION

TITLE: NUCLEOTIDE AND AMINO ACID SEQUENCES RELATING TO
RESPIRATORY DISEASES AND OBESITY

INVENTOR(S): Tim Keith et al.

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NUCLEOTIDE AND AMINO ACID SEQUENCES RELATING TO RESPIRATORY DISEASES AND OBESITY

RELATED APPLICATIONS

- 5 This application is a continuation-in-part of U.S. Application Serial Number 60/211,749, filed June 14, 2000, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

- 10 This invention relates to genes identified from human chromosome 12q23-qter, including Gene 454, Gene 561, and Gene 757, which are associated with asthma, obesity, inflammatory bowel disease, and other human diseases. The invention also relates to the nucleotide sequences of these genes, including genomic DNA sequences, cDNA sequences, and single nucleotide polymorphisms. The invention further relates to isolated
- 15 nucleic acids comprising these nucleotide sequences, and isolated polypeptides or peptides encoded thereby. Also related are expression vectors and host cells comprising the disclosed nucleic acids or fragments thereof, as well as antibodies that bind to the encoded polypeptides or peptides. The present invention further relates to ligands that modulate the
- 20 activity of the disclosed genes or gene products. In addition, the invention relates to diagnostics and therapeutics for various diseases, including asthma, utilizing the disclosed nucleic acids, polypeptides or peptides, antibodies, and/or ligands.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

- 25 Incorporated herein by reference in its entirety is a Sequence Listing, comprising SEQ ID NO:1 to SEQ ID NO:4687. The Sequence Listing is contained on a CD-ROM, three copies of which are filed, the Sequence Listing being in a computer-readable ASCII file named "Seqlist.txt", created

on June 7, 2001 and of 11,976 kilobyte in size, in IBM-PC Windows®NT v4.0 format.

BACKGROUND

Asthma has been linked to markers on human chromosome 12
5 (Wilson et al., 1998, *Genomics*, **53**: 251-259). In addition, obesity has been
linked to asthma (Wilson et al., 1999, *Arch. Intern. Med.* **159**: 2513-14). In
particular, chromosomal region 12q23-qter has been associated with a
variety of genetic disorders, including male germ cell tumors, histidinemia,
growth retardation with deafness and mental retardation, deficiency of Acyl-
10 CoA dehydrogenase, spinal muscular atrophy, Darier disease,
cardiomyopathy, Spinocerebellar ataxia-2, brachydactyly,
Mevalonicaciduria, Hyperimmunoglobulinemia D, Noonan syndrome-1,
Cardiofaciocutaneous syndrome, spinal muscular atrophy-4, tyrosinemia,
phenylketonuria, B-cell non-Hodgkin lymphoma, Ulnar-mammary syndrome,
15 Holt-Oram syndrome, Scapuloperoneal spinal muscular atrophy, alcohol
intolerance, MODY, Diabetes mellitus, noninsulin-dependent 2, and diabetes
mellitus insulin-dependent (See National Center for Biotechnology
Information: <http://www.ncbi.nlm.nih.gov/omim/>). The genes of this regions
are also associated with obesity, lung disease, particularly, inflammatory
20 lung disease phenotypes such as Chronic Obstructive Lung Disease
(COPD), Adult Respiratory Distress Syndrome (ARDS), and asthma.
However, few genes in chromosomal region 12q23-qter have been
discovered. Thus, there is a need in the art for the identification of specific
genes that are involved in these disorders. Identification and
25 characterization of such genes will allow the development of effective
diagnostics and therapeutic means to diagnose, prevent, and/or treat lung
related disorders, as well as the other diseases described herein.

SUMMARY OF THE INVENTION

This invention relates to isolated DNA comprising genes located on
30 chromosome 12q23-qter (see Table 4). In specific embodiments, the
invention relates to isolated nucleic acids comprising 12q23-qter genomic

sequences (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO: 156 to SEQ ID NO: 4973), cDNA and EST sequences (e.g., SEQ ID NO:1 to SEQ ID NO:92), BAC sequences (e.g., SEQ ID NO:156 to SEQ ID NO:693), BAC clones and contigs (e.g., SEQ ID NO: 694 to SEQ ID NO: 1265), direct
5 selected sequences (e.g., SEQ ID NO: 1266 to SEQ ID NO: 2052), clusters (e.g., SEQ ID NO: 2053 to SEQ ID NO: 4973), complementary sequences, sequence variants, or fragments thereof, as described herein. The present invention also encompasses nucleic acid probes or primers useful for assaying a biological sample for the presence or expression of 12q23-qter
10 genes.

The invention further encompasses nucleic acids variants comprising single nucleotide polymorphisms (SNPs) identified in several 12q23-qter genes (Table 10; Figures 7A-7H; Figures 9A-9F; Figures 27A-27K; and Figures 28A-28C). These include SNPs for gene 454 (SEQ ID NO: 19;
15 Figures 7A-7H), gene 561.1 (SEQ ID NO: 31; Figures 27A-27K), gene 561.2 (SEQ ID NO: 32; Figures 28A-28C), and gene 757 (SEQ ID NO: 90; Figures 9A-9F). SNPs can be used to diagnose diseases such as asthma, or to determine a genetic predisposition thereto. In addition, the present invention encompasses nucleic acids comprising alternate splicing variants
20 (e.g., SEQ ID NO:1 to SEQ ID NO:5; SEQ ID NO:17 to SEQ ID NO:18; SEQ ID NO:36 to SEQ ID NO:37; SEQ ID NO:43 to SEQ ID NO:44; and SEQ ID NO:80 to SEQ ID NO:81).

This invention also relates to vectors and host cells comprising vectors comprising the 12q23-qter nucleic acid sequences disclosed herein.
25 Such vectors can be used for nucleic acid preparations, including antisense nucleic acids, and for the expression of encoded polypeptides or peptides. Host cells can be prokaryotic or eukaryotic cells. In specific embodiments, an expression vector comprises a DNA sequence encoding the 12q23-qter polypeptide sequence (e.g., SEQ ID NO:93 to SEQ ID NO:155), sequence
30 variants, or fragments thereof, as described herein.

The present invention further relates to isolated 12q23-qter polypeptides and peptides. In specific embodiments, the polypeptides or peptides comprise the amino acid sequences encoded by the 12q23-qter genes (e.g., SEQ ID NO:93 to SEQ ID NO:155), sequence variants, or portions thereof, as described herein. In addition, this invention encompasses isolated fusion proteins comprising 12q23-qter polypeptides or peptides.

The present invention also relates to isolated antibodies, including monoclonal and polyclonal antibodies, and antibody fragments, that are specifically reactive with the 12q23-qter polypeptides, fusion proteins, or variants, or portions thereof, as disclosed herein. In specific embodiments, monoclonal antibodies are prepared to be specifically reactive with a 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155) or peptides, or sequence variants thereof.

In addition, the present invention relates to methods of obtaining 12q23-qter polynucleotides and polypeptides, variant sequences, or fragments thereof, as disclosed herein. Also related are methods of obtaining antibodies and antibody fragments that bind to 12q23-qter polypeptides, variant sequences, or fragments thereof. The present invention also encompasses methods of obtaining 12q23-qter ligands, e.g., agonists, antagonists, inhibitors, and binding factors. Such ligands can be used as therapeutics for asthma and related diseases.

The present invention also relates to diagnostic methods and kits utilizing obtaining 12q23-qter (wild-type, mutant, or variant) nucleic acids, polypeptides, antibodies, or functional fragments thereof. Such factors can be used, for example, in diagnostic methods and kits for measuring expression levels of obtaining 12q23-qter gene expression, and to screen for various obtaining 12q23-qter-related diseases, especially asthma. In addition, the nucleic acids described herein can be used to identify chromosomal abnormalities affecting 12q23-qter genes, and to identify

allelic variants or mutations of 12q23-qter genes in an individual or population.

The present invention further relates to methods and therapeutics for the treatment of various diseases, including asthma. In various
5 embodiments, therapeutics comprising the disclosed 12q23-qter nucleic acids, polypeptides, antibodies, ligands, or variants, derivatives, or portions thereof, are administered to a subject to treat, prevent, or ameliorate asthma. Specifically related are therapeutics comprising 12q23-qter antisense nucleic acids, monoclonal antibodies, and gene therapy vectors.
10 Such therapeutics can be administered alone, or in combination with one or more asthma treatments.

In addition, this invention relates to non-human transgenic animals and cell lines comprising one or more of the disclosed 12q23-qter nucleic acids, which can be used for drug screening, protein production, and other
15 purposes. Also related are non-human knock-out animals and cell lines, wherein one or more endogenous 12q23-qter genes (i.e., orthologs), or portions thereof, are deleted or replaced by marker genes.

This invention further relates to methods of identifying proteins that are candidates for being involved in asthma (i.e., a "candidate protein").
20 Such proteins are identified by a method comprising: 1) identifying a protein in a first individual having the asthma phenotype; 2) identifying a protein in a second individual not having the asthma phenotype; and 3) comparing the protein of the first individual to the protein of the second individual, wherein
25 a) the protein that is present in the second individual but not the first individual is the candidate protein; or b) the protein that is present in a higher amount in the second individual than in the first individual is the candidate protein; or c) the protein that is present in a lower amount in the second individual than in the first individual is the candidate protein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1D show the plot of multipoint LOD score against the map location of the markers along chromosome 12 for four phenotypes: asthma, bronchial hyper-responsiveness, total IgE, and specific IgE.

5 Figures 2A-2P show genes mapped to the 12q23-qter interval determined from information that is curated by the National Center for Biotechnology Information, "NCBI" (<http://www.ncbi.nlm.nih.gov/genemap/>). This particular information contains genes mapped against the Gene Bridge (GB) 4 panel.

10 Figures 3A-3G show genes mapped to the 12q23-qter interval determined from information that is curated by NCBI (<http://www.ncbi.nlm.nih.gov/genemap/>). This particular information contains genes mapped against the Gene Bridge (GB) 3 panel.

15 Figure 4 shows the integration of the Marshfield Center for Medical Genetics (<http://www.marshmed.org/genetics/>) genetic map with GeneMap99 from NCBI. The regions of study mentioned above are indicated at the top of the figure.

Figures 5A-5I show the BAC/STS content contig map for chromosome 12.

20 Figures 6A-6U show the results of Northern blot analysis of the Genes of 12q23-qter in various tissues.

Figures 7A-7H show the cDNA sequence and amino acid sequence of Gene 454 with the corresponding SNPs underlined.

25 Figure 8 shows the results of RT-PCR analysis of Gene 561.1 and Gene 561.2.

Figures 9A-9F show the cDNA sequence and amino acid sequence of Gene 757 with the corresponding SNPs underlined.

Figure 10 shows the domain structure of Gene 454 and the exon location of the corresponding SNPs.

Figure 11 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

Figure 12 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

Figure 13 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (BHR($\text{PC}_{20} \leq 16\text{mg/ml}$) and asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

Figure 14 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (BHR ($\text{PC}_{20} \leq 16\text{mg/ml}$) and asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

Figure 15 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (total IgE and asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

Figure 16 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (total IgE and asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

Figure 17 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (specific IgE and asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

Figure 18 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of SNP allele frequencies in cases (specific IgE and asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

Figure 19 shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

5 **Figure 20** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

10 **Figure 21** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (BHR ($\text{PC}_{20} \leq 16\text{mg/ml}$) and asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

15 **Figure 22** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (BHR ($\text{PC}_{20} \leq 16\text{mg/ml}$) and asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

20 **Figure 23** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (total IgE and asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

25 **Figure 24** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (total IgE and asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

30 **Figure 25** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (specific IgE and asthma) and controls in the combined population against the relative location (Kb) of SNPs along chromosome 12.

30 **Figure 26** shows the significance ($-\log_{10}(\text{p-value})$) for the comparison of haplotype frequencies (2-SNP-at-a-time) in cases (specific IgE and

asthma) and controls in the US and UK populations against the relative location (Kb) of SNPs along chromosome 12.

Figures 27A-27K show the cDNA sequence and amino acid sequence of Gene 561.1 with the corresponding SNPs underlined.

- 5 Figures 28A-28C show the cDNA sequence and amino acid sequence of Gene 561.2 with the corresponding SNPs underlined.

DETAILED DESCRIPTION OF THE INVENTION

- Chromosome 12q23-qter genes were isolated by narrowly defining the region of chromosome 12q23-qter that showed association with asthma.
- 10 Chromosome 12q23-qter genes have been implicated in other diseases, including obesity. Bronchial asthma, furthermore, has been linked to intestinal conditions such as inflammatory bowel disease (B. Wallaert et al., 1995, *J. Exp. Med.* 182:1897-1904). Thus, there was a need to identify and isolate the gene(s) associated with this region of human chromosome 12.

- 15 To aid in the understanding of the specification and claims, the following definitions are provided.

DEFINITIONS

- "Disorder region" refers to a portion of the human chromosome 12 bounded by the markers D12S2070 to the 12q telomere. A "disorder-associated" nucleic acid or "disorder-associated" polypeptide sequence refers to a nucleic acid sequence that maps to region 12q23-qter and polypeptides encoded thereby. For nucleic acid sequences, this encompasses sequences that are homologous or complementary to the reference sequence, as well as "sequence-conservative variants" and
- 20 "function-conservative variants." For polypeptide sequences, this encompasses "function-conservative variants." Also encompassed are naturally-occurring mutations associated with respiratory diseases including, but not limited to, asthma and atopy, as well as other diseases arising from mutations in this region including those described in detail herein. These
- 25 mutations are not limited to mutations that cause inappropriate expression
- 30

(e.g., lack of expression, over-expression, and expression in an inappropriate tissue type).

"Sequence-conservative" variants are those in which a change of one or more nucleotides in a given codon position results in no alteration in the amino acid encoded at that position (i.e., silent mutations). "Function-conservative" variants are those in which a change in one or more nucleotides in a given codon position results in a polypeptide sequence in which a given amino acid residue in a polypeptide has been changed without substantially altering the overall conformation and function of the native polypeptide, including, but not limited to, replacement of an amino acid with one having similar physico-chemical properties (such as, for example, acidic, basic, hydrophobic, and the like). "Function-conservative" variants also include analogs of a given polypeptide and any polypeptides that have the ability to elicit antibodies specific to a designated polypeptide.

"Nucleic acid or "polynucleotide" as used herein refers to purine- and pyrimidine-containing polymers of any length, either polyribonucleotides or polydeoxyribonucleotide or mixed polyribo-polydeoxyribonucleotides. This includes single-and double-stranded molecules, i.e., DNA-DNA, DNA-RNA and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid backbone. This also includes nucleic acids containing modified bases.

A "coding sequence" or a "protein-coding sequence" is a polynucleotide sequence capable of being transcribed into mRNA and/or capable of being translated into a polypeptide. The boundaries of the coding sequence are typically determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus.

As used herein, the "reference sequence" refers to the sequence used to compare individuals in identifying single nucleotide polymorphisms and the like. "Variant" sequences refer to nucleotide sequences (and in some cases, the encoded amino acid sequences) that differ from the reference sequence(s) at one or more positions. Non-limiting examples of

variant sequences include the disclosed single nucleotide polymorphisms (SNPs), alternate splice variants, and the amino acid sequences encoded by these variants.

"Expressed Sequence Tag (EST)" is a nucleic acid that encodes for a
5 portion of or a full-length protein sequence.

"12q23-qter genes" and "12q23-qter nucleic acids" include the genes and EST's shown in Figures 2A-2P and Figures 3A-3G, as well as the sequences listed in Table 4 (i.e., Gene 214, Gene 215, Gene 224, Gene 266, Gene 283, Gene 292, Gene 298, Gene 321, Gene 399, Gene 422,
10 Gene 436, Gene 454, Gene 515, Gene 536, Gene 543, Gene 548, Gene 549, Gene 550, Gene 551, Gene 553, Gene 555, Gene 558, Gene 559, Gene 561, Gene 562, Gene 563, Gene 564, Gene 566, Gene 567, Gene 570, Gene 571, Gene 572, Gene 575, Gene 577, Gene 579, Gene 580, Gene 581, Gene 583, Gene 584, Gene 586, Gene 587, Gene 589, Gene
15 590, Gene 592, Gene 593, Gene 594, Gene 595, Gene 596, Gene 601, Gene 603, Gene 604, Gene 605, Gene 606, Gene 608, Gene 611, Gene 615, Gene 617, Gene 618, Gene 620, Gene 621, Gene 622, Gene 690, Gene 692, Gene 693, Gene 694, Gene 695, Gene 697, Gene 698, Gene 699, Gene 702, Gene 705, Gene 707, Gene 722, Gene 748, Gene 749,
20 Gene 751, Gene 752, Gene 753, Gene 754, Gene 756, Gene 757, Gene 835, and Gene 848).

"12q23q-qter proteins" and "12q23q-qter polypeptides" include the polypeptide sequences encoded by the genes listed in Table 4.

A "complement" of a nucleic acid sequence as used herein refers to
25 the "antisense" sequence that participates in Watson-Crick base-pairing with the original sequence.

A "probe" refers to a nucleic acid or oligonucleotide that forms a hybrid structure with a sequence in a target region due to complementarity of at least one sequence in the probe with a sequence in the target region.

30 Nucleic acids are "hybridizable" to each other when at least one strand of nucleic acid can anneal to another nucleic acid strand under

defined stringency conditions. As is well known in the art, stringency of hybridization is determined, e.g., by (a) the temperature at which hybridization and/or washing is performed, and (b) the ionic strength and polarity (e.g., formamide) of the hybridization and washing solutions, as well as other parameters. Hybridization requires that the two nucleic acids contain substantially complementary sequences; depending on the stringency of hybridization, however, mismatches may be tolerated. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarity, variables well known in the art.

"Gene" refers to a DNA sequence that encodes through its template or messenger RNA a sequence of amino acids characteristic of a specific peptide, polypeptide, or protein. The term "gene" as used herein with reference to genomic DNA includes intervening, non-coding regions, as well as regulatory regions, and can include 5' and 3' ends.

"Gene sequence" refers to a DNA molecule, including a DNA molecule that contains a non-transcribed or non-translated sequence. The term is also intended to include any combination of gene(s), gene fragment(s), non-transcribed sequence(s), or non-translated sequence(s) that are present on the same DNA molecule.

A gene sequence is "wild-type" if such sequence is usually found in individuals unaffected by the disease or condition of interest. However, environmental factors and other genes can also play an important role in the ultimate determination of the disease. In the context of complex diseases involving multiple genes ("oligogenic disease"), the "wild type", or normal sequence can also be associated with a measurable risk or susceptibility, receiving its reference status based on its frequency in the general population. As used herein, "wild-type" refers to the reference sequence. The wild-type sequences are used to identify the variants (single nucleotide polymorphisms) described in detail herein.

A gene sequence is a "mutant" sequence if it differs from the wild-type sequence. For example, a Gene 454 nucleic acid containing a single nucleotide polymorphism is a mutant sequence. In some cases, the individual carrying such gene has increased susceptibility toward the disease or condition of interest. In other cases, the "mutant" sequence might also refer to a sequence that decreases the susceptibility toward a disease or condition of interest, and thus acting in a protective manner. Also a gene is a "mutant" gene if too much ("overexpressed") or too little ("underexpressed") of such gene is expressed in the tissues in which such gene is normally expressed, thereby causing the disease or condition of interest.

"cDNA" refers to complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase). Thus, a "cDNA clone" means a duplex DNA sequence complementary to an RNA molecule of interest, carried in a cloning vector or PCR amplified. This term includes genes from which the intervening sequences have been removed.

"Recombinant DNA" means a molecule that has been recombined by *in vitro* splicing/and includes cDNA or a genomic DNA sequence.

"Cloning" refers to the use of *in vitro* recombination techniques to insert a particular gene or other DNA sequence into a vector molecule. In order to successfully clone a desired gene, it is necessary to use methods for generating DNA fragments, for joining the fragments to vector molecules, for introducing the composite DNA molecule into a host cell in which it can replicate, and for selecting the clone having the target gene from amongst the recipient host cells.

"cDNA library" refers to a collection of recombinant DNA molecules containing cDNA inserts, which together comprise the entire genome of an organism. Such a cDNA library can be prepared by methods known to one skilled in the art and described by, for example, Cowell and Austin, 1997, "cDNA Library Protocols," Methods in Molecular Biology. Generally, RNA is

first isolated from the cells of an organism from whose genome it is desired to clone a particular gene.

The term "vector" as used herein refers to a nucleic acid molecule capable of replicating another nucleic acid to which it has been linked. A
5 vector, for example, can be a plasmid.

"Cloning vector" refers to a plasmid or phage DNA or other DNA sequence that is able to replicate in a host cell. The cloning vector is characterized by one or more endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without loss of an
10 essential biological function of the DNA, which may contain a marker suitable for use in the identification of transformed cells.

"Expression vector" refers to a vehicle or vector similar to a cloning vector but which is capable of expressing a nucleic acid sequence that has been cloned into it, after transformation into a host. A nucleic acid sequence
15 is "expressed" when it is transcribed to yield an mRNA sequence. In most cases, this transcript will be translated to yield amino acid sequence. The cloned gene is usually placed under the control of (i.e., operably linked to) an expression control sequence.

"Expression control sequence" or "regulatory sequence" refers to a
20 nucleotide sequence that controls or regulates expression of structural genes when operably linked to those genes. These include, for example, the lac systems, the trp system, major operator and promoter regions of the phage lambda, the control region of fd coat protein and other sequences known to control the expression of genes in prokaryotic or eukaryotic cells.
25 Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host, and may contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements and/or translational initiation and termination sites.

30 "Operably linked" means that the promoter controls the initiation of expression of the gene. A promoter is operably linked to a sequence of

proximal DNA if upon introduction into a host cell the promoter determines the transcription of the proximal DNA sequence(s) into one or more species of RNA. A promoter is operably linked to a DNA sequence if the promoter is capable of initiating transcription of that DNA sequence.

5 "Host" includes prokaryotes and eukaryotes. The term includes an organism or cell that is the recipient of a replicable expression vector.

The introduction of the nucleic acids into the host cell by any method known in the art, including those described herein, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids
10 described above are meant to also include the progeny of such cells.

"Amplification of nucleic acids" refers to methods such as polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. These methods are well known in the art and described, for example, in U.S.
15 Patent Nos. 4,683,195 and 4,683,202. Reagents and hardware for conducting PCR are commercially available. Primers useful for amplifying sequences from the disorder region are preferably complementary to, and preferably hybridize specifically to, sequences in the 12q23-qter region or in regions that flank a target region therein. Chromosome 12q23-qter genes
20 generated by amplification may be sequenced directly. Alternatively, the amplified sequence(s) may be cloned prior to sequence analysis.

A nucleic acid or fragment thereof is "substantially homologous" or "substantially similar" to another if, when optimally aligned (with appropriate nucleotide insertions and/or deletions) with the other nucleic acid (or its
25 complementary strand), there is nucleotide sequence identity in at least 60% of the nucleotide bases, usually at least 70%, more usually at least 80%, preferably at least 90%, and more preferably at least 95-98% of the nucleotide bases.

Alternatively, substantial homology or similarity exists when a nucleic
30 acid or fragment thereof will hybridize, under selective hybridization conditions, to another nucleic acid (or a complementary strand thereof).

Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least 55% homology over a stretch of at least nine or more nucleotides, preferably at least 65%, more preferably at least 75%, and most preferably at least 90% (see, M. Kanehisa, 1984, *Nucl. Acids Res.* 11:203-213). The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least 14 nucleotides, usually at least 20 nucleotides, more usually at least 24 nucleotides, typically at least 28 nucleotides, more typically at least 32 nucleotides, and preferably at least 36 or more nucleotides.

Nucleic acids referred to herein as "isolated" are nucleic acids separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (e.g., as it exists in cells or in a mixture of nucleic acids such as a library), and may have undergone further processing. "Isolated", as used herein, refers to nucleic or amino acid sequences that are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. "Isolated" nucleic acids (polynucleotides) include nucleic acids obtained by methods described herein, similar methods or other suitable methods, including essentially pure nucleic acids, nucleic acids produced by chemical synthesis, by combinations of biological and chemical methods, and recombinant nucleic acids which are isolated. Nucleic acids referred to herein as "recombinant" are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures which rely upon a method of artificial replication, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. "Recombinant" nucleic acids are also those that result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction to the cells of nucleic acids designed to allow or make probable a desired recombination event.

Portions of the isolated nucleic acids which code for polypeptides having a certain function can be identified and isolated by, for example, the method of Jasin, M., et al., U.S. Patent No. 4,952,501.

In the context of this invention, the term "oligonucleotide" refers to
5 naturally-occurring species or synthetic species formed from naturally-occurring subunits or their close homologs. The term may also refer to moieties that function similarly to oligonucleotides, but have non-naturally-occurring portions. Thus, oligonucleotides may have altered sugar moieties or inter-sugar linkages. Exemplary among these are phosphorothioate and
10 other sulfur containing species which are known in the art.

As used herein, the terms "protein" and "polypeptide" are synonymous. "Peptides" are defined as fragments or portions of polypeptides, preferably fragments or portions having at least one functional activity (e.g., proteolysis, adhesion, fusion, antigenic, or intracellular activity)
15 as the complete polypeptide sequence.

As used herein, "isolated" proteins or polypeptides are proteins or polypeptides purified to a state beyond that in which they exist in cells. In a preferred embodiment, they are at least 10% pure; i.e., most preferably they are substantially purified to 80 or 90% purity. "Isolated" proteins or
20 polypeptides include proteins or polypeptides obtained by methods described *infra*, similar methods or other suitable methods, and include essentially pure proteins or polypeptides, proteins or polypeptides produced by chemical synthesis or by combinations of biological and chemical methods, and recombinant proteins or polypeptides which are isolated.
25 Proteins or polypeptides referred to herein as "recombinant" are proteins or polypeptides produced by the expression of recombinant nucleic acids.

A "portion" as used herein with regard to a protein or polypeptide, refers to fragments of that protein or polypeptide. The fragments can range in size from 5 amino acid residues to all but one residue of the entire protein
30 sequence. Thus, a portion or fragment can be at least 5, 5-50, 50-100, 100-200, 200-400, 400-800, or more consecutive amino acid residues of a

chromosome 12q23-qter protein or polypeptide, for example, SEQ ID NO:93 to SEQ ID NO:155, or variants thereof.

5 The term "immunogenic", refers to the ability of a molecule (e.g., a polypeptide or peptide) to elicit a humoral and/or cellular immune response in a host animal.

The term "antigenic" refers to the ability of a molecule (e.g., a polypeptide or peptide) to bind to its specific antibody with sufficiently high affinity to form a detectable antigen-antibody complex.

10 "Antibodies" refer to polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, that can bind to asthma proteins and fragments thereof or to nucleic acid sequences from the 12q23-qter region, particularly from the asthma locus or a portion thereof. The term antibody is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a
15 plurality of different molecular entities.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a 12q23-qter polypeptide or
20 peptide. A monoclonal antibody composition thus typically displays a single binding affinity for a particular 12q23-qter polypeptide or peptide with which it immunoreacts.

The term "ligand" as used herein describes any molecule, protein, peptide, or compound with the capability of directly or indirectly altering the
25 physiological function, stability, or levels of a polypeptide.

A "sample" as used herein refers to a biological sample, such as, for example, tissue or fluid isolated from an individual (including, without limitation, plasma, serum, cerebrospinal fluid, lymph, tears, saliva, milk, pus, and tissue exudates and secretions) or from *in vitro* cell culture constituents,
30 as well as samples obtained from, for example, a laboratory procedure.

As used herein, the term "ortholog" denotes a gene or polypeptide obtained from one species that has homology to an analogous gene or polypeptide from a different species. This is in contrast to "paralog", which denotes a gene or polypeptide obtained from a given species that has
 5 homology to a distinct gene or polypeptide from that same species.

Standard reference works setting forth the general principles of recombinant DNA technology include J. Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; P.B. Kaufman et al., (eds), 1995, *Handbook*
 10 *of Molecular and Cellular Methods in Biology and Medicine*, CRC Press, Boca Raton; M.J. McPherson (ed), 1991, *Directed Mutagenesis: A Practical Approach*, IRL Press, Oxford; J. Jones, 1992, *Amino Acid and Peptide Synthesis*, Oxford Science Publications, Oxford; B.M. Austen and O.M.R. Westwood, 1991, *Protein Targeting and Secretion*, IRL Press, Oxford; D.N
 15 Glover (ed), 1985, *DNA Cloning*, Volumes I and II; M.J. Gait (ed), 1984, *Oligonucleotide Synthesis*; B.D. Hames and S.J. Higgins (eds), 1984, *Nucleic Acid Hybridization*; Wu and Grossman (eds), *Methods in Enzymology* (Academic Press, Inc.), Vol. 154 and Vol. 155; Quirke and Taylor (eds), 1991, *PCR-A Practical Approach*; Hames and Higgins (eds),
 20 1984, *Transcription and Translation*; R.I. Freshney (ed), 1986, *Animal Cell Culture; Immobilized Cells and Enzymes*, 1986, IRL Press; Perbal, 1984, *A Practical Guide to Molecular Cloning*; J. H. Miller and M. P. Calos (eds), 1987, *Gene Transfer Vectors for Mammalian Cells*, Cold Spring Harbor Laboratory Press; M.J. Bishop (ed), 1998, *Guide to Human Genome*
 25 *Computing*, 2d Ed., Academic Press, San Diego, CA; L.F. Peruski and A.H. Peruski, 1997, *The Internet and the New Biology: Tools for Genomic and Molecular Research*, American Society for Microbiology, Washington, D.C.

Standard reference works setting forth the general principles of immunology include S. Sell, 1996, *Immunology, Immunopathology &*
 30 *Immunity*, 5th Ed., Appleton & Lange, Publ., Stamford, CT; D. Male et al., 1996, *Advanced Immunology*, 3d Ed., Times Mirror Int'l Publishers Ltd.,

Publ., London; D.P. Stites and A.I. Terr, 1991, *Basic and Clinical Immunology*, 7th Ed., Appleton & Lange, Publ., Norwalk, CT; and A.K. Abbas et al., 1991, *Cellular and Molecular Immunology*, W. B. Saunders Co., Publ., Philadelphia, PA. Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention; however, preferred materials and/or methods are described. Materials, reagents, and the like to which reference is made in the following description and examples are generally obtainable from commercial sources, and specific vendors are cited herein.

10 **NUCLEIC ACIDS**

The present invention relates to nucleic acids from chromosome 12q23-qter genes (Table 4; e.g., SEQ ID NO: 1 to SEQ ID NO:92, genomic DNA within BAC end sequences (e.g., SEQ ID NO:156 to SEQ ID NO:693), and genomic DNA of BAC sequences (e.g., SEQ ID NO:694 to SEQ ID NO:979), direct selected sequences (e.g., SEQ ID NO:980 to SEQ ID NO:1766), clusters (e.g., SEQ ID NO:1767 to SEQ ID NO:4687), RNA, fragments of the genomic, cDNA, or RNA nucleic acids comprising 20, 40, 60, 100, 200, 500 or more contiguous nucleotides, and the complements thereof. Closely related variants are also included as part of this invention, as well as recombinant nucleic acids comprising at least 50, 60, 70, 80, or 90% of the nucleic acids described above which would be identical to nucleic acids from chromosome 12q23-qter genes except for one or a few substitutions, deletions, or additions.

Further, the nucleic acids of this invention include the adjacent chromosomal regions of chromosome 12q23-qter genes required for accurate expression of the respective gene. In a preferred embodiment, the present invention is directed to at least 15 contiguous nucleotides of the nucleic acid sequence of any of SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687. More particularly, embodiments of this invention include the BAC clones containing segments of chromosome 12q23-qter genes including RPCI-11_0899A17, RPCI-11_0666B20, RPCI-

11_0723P10, RPCI-11_0831E18, RPCI-11_0932D22, and RPCI-11_0702C13. A preferred embodiment is the nucleotide sequence of the BAC clones consisting of SEQ ID NO:694 to SEQ ID NO:979 and those listed in Table 3. Another embodiment is the nucleotide sequence of the BAC end sequences of SEQ ID NO:156 to SEQ ID NO:693.

The invention also relates to direct selected clones and EST's from the 12q23-qter (e.g., SEQ ID NO:1 to SEQ ID NO:92). In a preferred embodiment, the invention relates to clusters of nucleic acids combining the direct selected clones with EST's homologous to the BAC sequences and BAC end sequences (SEQ ID NO:1675 to SEQ ID NO:4594).

The invention also concerns the use of the nucleotide sequence of the nucleic acids of this invention to identify DNA probes for genes of 12q23-qter (SEQ ID NO:1 to SEQ ID NO:92), BAC end sequences (SEQ ID NO:156 to SEQ ID NO:693), BACs (SEQ ID NO:694 to SEQ ID NO:979), direct selected clones (SEQ ID NO:980 to SEQ ID NO:1766), and sequence clusters (SEQ ID NO:1767 to SEQ ID NO:4687), PCR primers to amplify the genes of 12q23-qter, nucleotide polymorphisms (Table 10), and regulatory elements of the genes of 12q23-qter.

This invention further relates to methods of using isolated and/or recombinant 12q23-qter nucleic acids (DNA or RNA) that are characterized by their ability to hybridize to (a) a nucleic acid encoding a protein or polypeptide, such as a nucleic acid having any of the sequences SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687, or (b) a fragment of the foregoing (e.g., any of the nucleotide sequences set forth in Tables 8, 9, 11A and 11B). For example, a fragment can comprise the minimum nucleotides of a chromosome 12q23-qter protein required to encode a functional chromosome 12q23-qter protein, or the minimum nucleotides to encode a polypeptide having the amino acid sequence of SEQ ID NO:93 to SEQ ID NO:155, or to encode a functional equivalent thereof. A functional equivalent can include a polypeptide, which, when incorporated into a cell, has all or part of the activity of a chromosome

12q23-qter protein. A functional equivalent of a chromosome 12q23-qter protein, therefore, would have a similar amino acid sequence (at least 65% sequence identity) and similar characteristics to, or perform in substantially the same way as a chromosome 12q23-qter protein. A nucleic acid which
5 hybridizes to a nucleic acid encoding a chromosome 12q23-qter protein or polypeptide, such as SEQ ID NO:93 to SEQ ID NO:155, can be double- or single-stranded. Hybridization to DNA, such as DNA having a sequence set forth in SEQ ID NO:1 to SEQ ID NO:92, SEQ ID NO:156 to SEQ ID NO:4687, Tables 8, 9, 11A, and 11B, includes hybridization to the strand
10 shown, or to the complementary strand.

The sequences of the present invention may be derived from a variety of sources including DNA, cDNA, synthetic DNA, synthetic RNA, or combinations thereof. Such sequences may comprise genomic DNA, which may or may not include naturally occurring introns. Moreover, such genomic
15 DNA may be obtained in association with promoter regions or poly (A) sequences. The sequences, genomic DNA, or cDNA may be obtained in any of several ways. Genomic DNA can be extracted and purified from suitable cells by means well known in the art. Alternatively, mRNA can be isolated from a cell and used to produce cDNA by reverse transcription or
20 other means.

The present invention also relates to nucleic acids that encode a polypeptide having the amino acid sequence of any one of SEQ ID NO:93 to SEQ ID NO:155, or functional equivalents thereof. A functional equivalent of a 12q23-qter protein includes fragments or variants that perform at least on
25 characteristic function of the 12q23-qter protein (e.g., antigenic or intracellular activity). Preferably, a functional equivalent will share at least 65% sequence identity with the 12q23-qter polypeptide.

Sequence identity calculations can be performed using computer programs, hybridization methods, or calculations. Preferred computer
30 program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package,

BLASTN, BLASTX, TBLASTX, and FASTA (J. Devereux et al., 1984, *Nucleic Acids Research* 12(1):387; S.F. Altschul et al., 1990, *J. Molec. Biol.* 215:403-410; W. Gish and D.J. States, 1994, *Nature Genet.* 3:266-272; W.R. Pearson and D.J. Lipman, 1988, *Proc Natl. Acad. Sci. USA* 85(8):2444-8). The BLAST programs are publicly available from NCBI and other sources . The well-known Smith Waterman algorithm may also be used to determine identity.

For example, nucleotide sequence identity can be determined by comparing a query sequences to sequences in publicly available sequence databases (NCBI) using the BLASTN2 algorithm (S.F. Altschul et al., 1997, *Nucl. Acids Res.*, 25:3389-3402). The parameters for a typical search are: E = 0.05, v = 50, B = 50, wherein E is the expected probability score cutoff, V is the number of database entries returned in the reporting of the results, and B is the number of sequence alignments returned in the reporting of the results (S.F. Altschul et al., 1990, *J. Mol. Biol.*, 215:403-410).

In another approach, nucleotide sequence identity can be calculated using the following equation: % identity = (number of identical nucleotides) / (alignment length in nucleotides) * 100. For this calculation, alignment length includes internal gaps but not includes terminal gaps. Alternatively, nucleotide sequence identity can be determined experimentally using the specific hybridization conditions described below.

In accordance with the present invention, polynucleotide alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, insertion, or modification (e.g., via RNA or DNA analogs). Alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Alterations of a polynucleotide sequence of any one of SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687 may create nonsense, missense, or frameshift mutations in this

coding sequence, and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Such altered nucleic acids, including DNA or RNA, can be detected and isolated by hybridization under high stringency conditions or moderate stringency conditions, for example, which are chosen to prevent hybridization of nucleic acids having non-complementary sequences. "Stringency conditions" for hybridizations is a term of art which refers to the conditions of temperature and buffer concentration which permit hybridization of a particular nucleic acid to another nucleic acid in which the first nucleic acid may be perfectly complementary to the second, or the first and second may share some degree of complementarity which is less than perfect.

For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained in F.M. Ausubel et al. (eds), 1995, *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., New York, NY, the teachings of which are hereby incorporated by reference. In particular, see pages 2.10.1-2.10.16 (especially pages 2.10.8-2.10.11) and pages 6.3.1-6.3.6. The exact conditions which determine the stringency of hybridization depend not only on ionic strength, temperature and the concentration of destabilizing agents such as formamide, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, high or moderate stringency conditions can be determined empirically.

By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize with the most similar sequences in the sample can be determined. Preferably the

hybridizing sequences will have 60-70% sequence identity, more preferably 70-85% sequence identity, and even more preferably 90-100% sequence identity.

Typically, the hybridization reaction is initially performed under conditions of low stringency, followed by washes of varying, but higher stringency. Reference to hybridization stringency, e.g., high, moderate, or low stringency, typically relates to such washing conditions. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid probe or primer and are typically classified by degree of stringency of the conditions under which hybridization is measured (Ausubel et al., 1995). For example, high stringency hybridization typically occurs at about 5-10% C below the T_m ; moderate stringency hybridization occurs at about 10-20% below the T_m ; and low stringency hybridization occurs at about 20-25% below the T_m . The melting temperature can be approximated by the formulas as known in the art, depending on a number of parameters, such as the length of the hybrid or probe in number of nucleotides, or hybridization buffer ingredients and conditions. As a general guide, T_m decreases approximately 1°C with every 1% decrease in sequence identity at any given SSC concentration. Generally, doubling the concentration of SSC results in an increase in T_m of ~17°C. Using these guidelines, the washing temperature can be determined empirically for moderate or low stringency, depending on the level of mismatch sought.

High stringency hybridization conditions are typically carried out at 65 to 68°C in 0.1 X SSC and 0.1% SDS. Highly stringent conditions allow hybridization of nucleic acid molecules having about 95 to 100% sequence identity. Moderate stringency hybridization conditions are typically carried out at 50 to 65°C in 1 X SSC and 0.1% SDS. Moderate stringency conditions allow hybridization of sequences having at least 80 to 95% nucleotide sequence identity. Low stringency hybridization conditions are typically carried out at 40 to 50°C in 6 X SSC and 0.1% SDS. Low stringency hybridization conditions allow detection of specific hybridization of

nucleic acid molecules having at least 50 to 80% nucleotide sequence identity.

For example, high stringency conditions can be attained by hybridization in 50% formamide, 5 X Denhardt's solution, 5 X SSPE or SSC (1 X SSPE buffer comprises 0.15 M NaCl, 10 mM Na₂HPO₄, 1 mM EDTA; 1 X SSC buffer comprises 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 0.2% SDS at about 42°C, followed by washing in 1 X SSPE or SSC and 0.1% SDS at a temperature of at least 42°C, preferably about 55°C, more preferably about 65°C. Moderate stringency conditions can be attained, for example, by hybridization in 50% formamide, 5 X Denhardt's solution, 5 X SSPE or SSC, and 0.2% SDS at 42°C to about 50°C, followed by washing in 0.2 X SSPE or SSC and 0.2% SDS at a temperature of at least 42°C, preferably about 55°C, more preferably about 65°C. Low stringency conditions can be attained, for example, by hybridization in 10% formamide, 5 X Denhardt's solution, 6 X SSPE or SSC, and 0.2% SDS at 42°C, followed by washing in 1 X SSPE or SSC, and 0.2% SDS at a temperature of about 45°C, preferably about 50°C in 4 X SSC at 60°C for 30 min.

High stringency hybridization procedures typically (1) employ low ionic strength and high temperature for washing, such as 0.015 M NaCl/0.0015 M sodium citrate, pH 7.0 (0.1 X SSC) with 0.1% sodium dodecyl sulfate (SDS) at 50°C; (2) employ during hybridization 50% (vol/vol) formamide with 5 X Denhardt's solution (0.1% weight/volume highly purified bovine serum albumin/0.1% wt/vol Ficoll/0.1% wt/vol polyvinylpyrrolidone), 50 mM sodium phosphate buffer at pH 6.5 and 5 X SSC at 42°C; or (3) employ hybridization with 50% formamide, 5 X SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 X Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 X SSC and 0.1% SDS.

In one particular embodiment, high stringency hybridization conditions may be attained by:

--Prehybridization treatment of the support (e.g., nitrocellulose filter or nylon membrane), to which is bound the nucleic acid capable of hybridizing with any of the sequences of the invention, is carried out at 65°C for 6 hr with a solution having the following composition: 4 X SSC, 10 X Denhardt's
 5 (1 X Denhardt's comprises 1% Ficoll, 1% polyvinylpyrrolidone, 1% BSA (bovine serum albumin); 1 X SSC comprises of 0.15 M of NaCl and 0.015 M of sodium citrate, pH 7);

--Replacement of the pre-hybridization solution in contact with the support by a buffer solution having the following composition: 4 X SSC, 1 X
 10 Denhardt's, 25 mM NaPO₄, pH 7, 2 mM EDTA, 0.5% SDS, 100 µg/ml of sonicated salmon sperm DNA containing a nucleic acid derived from the sequences of the invention as probe, in particular a radioactive probe, and previously denatured by a treatment at 100°C for 3 min;

--Incubation for 12 hr at 65°C;

15 --Successive washings with the following solutions: 1) four washings with 2 X SSC, 1 X Denhardt's, 0.5% SDS for 45 min at 65°C; 2) two washings with 0.2 X SSC, 0.1 X SSC for 45 min at 65°C; and 3) 0.1 x SSC, 0.1% SDS for 45 min at 65°C.

Additional examples of high, medium, and low stringency conditions
 20 can be found in Sambrook et al., 1989. Exemplary conditions are also described in M.H. Krause and S.A. Aaronson, 1991, *Methods in Enzymology*, 200:546-556; Ausubel et al., 1995. It is to be understood that the low, moderate and high stringency hybridization/washing conditions may be varied using a variety of ingredients, buffers, and temperatures well
 25 known to and practiced by the skilled practitioner.

Isolated and/or recombinant nucleic acids that are characterized by their ability to hybridize to a) a nucleic acid encoding a chromosome 12q23-
 qter polypeptide, such as the nucleic acids depicted as SEQ ID NO:1 to SEQ ID NO:92; b) the complement of (a); c) or a portion of (a) or (b) (e.g.,
 30 under high or moderate stringency conditions), may further encode a protein or polypeptide having at least one function characteristic of a chromosome

12q23-qter polypeptide, such as Gene 702, a metalloprotease-like gene involved in inflammatory responses including tissue destruction and repair, or binding of antibodies that also bind to non-recombinant chromosome 12q23-qter proteins or polypeptides. The catalytic or binding function of a protein or polypeptide encoded by the hybridizing nucleic acid may be detected by standard enzymatic assays for activity or binding (e.g., assays that measure the binding of a transit peptide or a precursor, or other components of the translocation machinery). Enzymatic assays, complementation tests, or other suitable methods can also be used in procedures for the identification and/or isolation of nucleic acids which encode a polypeptide such as a polypeptide of the amino acid sequences SEQ ID NO:93 to SEQ ID NO:155, or a functional equivalent of these polypeptides. The antigenic properties of proteins or polypeptides encoded by hybridizing nucleic acids can be determined by immunological methods employing antibodies that bind to a chromosome 12q23-qter polypeptide such as immunoblot, immunoprecipitation and radioimmunoassay. PCR methodology, including RAGE (Rapid Amplification of Genomic DNA Ends), can also be used to screen for and detect the presence of nucleic acids which encode chromosome 12q23-qter gene-like proteins and polypeptides, and to assist in cloning such nucleic acids from genomic DNA. PCR methods for these purposes can be found in Innis, M.A., et al., 1990, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA., incorporated herein by reference.

It is understood that, as a result of the degeneracy of the genetic code, many nucleic acid sequences are possible which encode a chromosome 12q23-qter gene-like protein or polypeptide. Some of these will have little homology to the nucleotide sequences of any known or naturally-occurring chromosome 12q23-qter gene-like gene but can be used to produce the proteins and polypeptides of this invention by selection of combinations of nucleotide triplets based on codon choices. Such variants,

while not hybridizable to a naturally-occurring chromosome 12q23-qter gene, are contemplated within this invention.

Also encompassed by the present invention are alternate splice variants produced by differential processing of the primary transcript(s) from 12q23-qter genomic DNA. An alternate splice variant may comprise, for example, the sequence of any one of SEQ ID NO:1 to SEQ ID NO:5; SEQ ID NO:17 to SEQ ID NO:18; SEQ ID NO:36 to SEQ ID NO:37; SEQ ID NO:43 to SEQ ID NO:44; and SEQ ID NO:80 to SEQ ID NO:81. Alternate splice variants can also comprise other combinations of introns/exons of 12q23-qter genes, which can be determined by those of skill in the art. Alternate splice variants can be determined experimentally, for example, by isolating and analyzing cellular RNAs (e.g., Southern blotting or PCR), or by screening cDNA libraries using the 12q23-qter nucleic acid probes or primers described herein. In another approach, alternate splice variants can be predicted using various methods, computer programs, or computer systems available to practitioners in the field.

General methods for splice site prediction can be found in Nakata, 1985, *Nucleic Acids Res.* 13:5327-5340. In addition, splice sites can be predicted using, for example, the GRAIL™ (E.C. Uberbacher and R.J. Mural, 1991, *Proc. Natl. Acad. Sci. USA*, 88:11261-11265; E.C. Uberbacher, 1995, *Trends Biotech.*, 13:497-500; <http://grail.lsd.ornl.gov/grailexp>); GenView (L. Milanesi et al., 1993, *Proceedings of the Second International Conference on Bioinformatics, Supercomputing, and Complex Genome Analysis*, H.A. Lim et al. (eds), World Scientific Publishing, Singapore, pp. 573-588; http://l25.itba.mi.cnr.it/~webgene/wwwgene_help.html); SpliceView (<http://www.itba.mi.cnr.it/webgene>); and HSPL (V.V. Solovyev et al., 1994, *Nucleic Acids Res.* 22:5156-5163; V.V. Solovyev et al., 1994, "The Prediction of Human Exons by Oligonucleotide Composition and Discriminant Analysis of Spliceable Open Reading Frames," R. Altman et al. (eds), *The Second International conference on Intelligent systems for Molecular Biology*, AAAI Press, Menlo Park, CA, pp. 354-362; V.V. Solovyev

et al., 1993, "Identification Of Human Gene Functional Regions Based On Oligonucleotide Composition," L. Hunter et al. (eds), *In Proceedings of First International conference on Intelligent System for Molecular Biology*. Bethesda, pp. 371-379) computer systems.

- 5 Additionally, computer programs such as GeneParser (E.E. Snyder and G.D. Stormo, 1995, *J. Mol. Biol.* 248: 1-18; E.E. Snyder and G.D. Stormo, 1993, *Nucl. Acids Res.* 21(3): 607-613; <http://mcdb.colorado.edu/~eesnyder/GeneParser.html>); MZEF (M.Q. Zhang, 1997, *Proc. Natl. Acad. Sci. USA*, 94:565-568; <http://argon.cshl.org/genefinder>); MORGAN (S. Salzberg et al., 1998, *J. Comp. Biol.* 5:667-680; S. Salzberg et al. (eds), 1998, *Computational Methods in Molecular Biology*, Elsevier Science, New York, NY, pp. 187-203); VEIL (J. Henderson et al., 1997, *J. Comp. Biol.* 4:127-141); GeneScan (S. Tiwari et al., 1997, *CABIOS (Bioinformatics)* 13: 263-270); GeneBuilder (L. Milanesi et al., 1999, *Bioinformatics* 15:612-621); Eukaryotic GeneMark (J. Besemer et al., 1999, *Nucl. Acids Res.* 27:3911-3920); and FEXH (V.V. Solovyev et al., 1994, *Nucleic Acids Res.* 22:5156-5163). In addition, splice sites (i.e., former or potential splice sites) in cDNA sequences can be predicted using, for example, the RNASPL (V.V. Solovyev et al., 1994, *Nucleic Acids Res.* 22:5156-5163); or INTRON (A. Globek et al., 1991, INTRON version 1.1 manual, Laboratory of Biochemical Genetics, NIMH, Washington, D.C.) programs.
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- 20

The present invention also encompasses naturally-occurring polymorphisms of 12q23-qter genes. As will be understood by those in the art, the genomes of all organisms undergo spontaneous mutation in the course of their continuing evolution generating variant forms of gene sequences (Gusella, 1986, *Ann. Rev. Biochem.* 55:831-854). Restriction fragment length polymorphisms (RFLPs) include variations in DNA sequences that alter the length of a restriction fragment in the sequence (Botstein et al., 1980, *Am. J. Hum. Genet.* 32, 314-331). RFLPs have been widely used in human and animal genetic analyses (see WO 90/13668;

WO90/11369; Donis-Keller, 1987, *Cell* 51:319-337; Lander et al., 1989, *Genetics* 121: 85-99). Short tandem repeats (STRs) include tandem di-, tri- and tetranucleotide repeated motifs, also termed variable number tandem repeat (VNTR) polymorphisms. VNTRs have been used in identity and paternity analysis (U.S. Pat. No. 5,075,217; Armour et al., 1992, *FEBS Lett.* 307:113-115; Horn et al., WO 91/14003; Jeffreys, EP 370,719), and in a large number of genetic mapping studies.

Single nucleotide polymorphisms (SNPs) are far more frequent than RFLPS, STRs, and VNTRs. SNPs may occur in protein coding (e.g., exon), or non-coding (e.g., intron, 5'UTR, 3'UTR) sequences. SNPs in protein coding regions may comprise silent mutations that do not alter the amino acid sequence of a protein. Alternatively, SNPs in protein coding regions may produce conservative or non-conservative amino acid changes, described in detail below. In some cases, SNPs may give rise to the expression of a defective or other variant protein and, potentially, a genetic disease. SNPs within protein-coding sequences can give rise to genetic diseases, for example, in the β -globin (sickle cell anemia) and CFTR (cystic fibrosis) genes. In non-coding sequences, SNPs may also result in defective protein expression (e.g., as a result of defective splicing). Other single nucleotide polymorphisms have no phenotypic effects.

Single nucleotide polymorphisms can be used in the same manner as RFLPs and VNTRs, but offer several advantages. Single nucleotide polymorphisms tend to occur with greater frequency and are typically spaced more uniformly throughout the genome than other polymorphisms. Also, different SNPs are often easier to distinguish than other types of polymorphisms (e.g., by use of assays employing allele-specific hybridization probes or primers). In one embodiment of the present invention, a 12q23-qter nucleic acid contains at least one SNP as set forth in Table 10, Figures 7A-7H; Figures 9A-9F; Figures 27A-27K; and Figures 28A-28C, described herein. Various combinations of these SNPs are also encompassed by the invention. In a preferred aspect, a 12q23-qter SNP is

associated with a lung-related disorder, such as asthma. Nucleic acids comprising such SNPs can be used as diagnostic and/or therapeutic reagents.

5 The nucleic acid sequences of the present invention may be derived from a variety of sources including DNA, cDNA, synthetic DNA, synthetic RNA, or combinations thereof. Such sequences may comprise genomic DNA, which may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with promoter regions or poly(A)+ sequences. The sequences, genomic DNA, or cDNA may be
10 obtained in any of several ways. Genomic DNA can be extracted and purified from suitable cells by means well known in the art. Alternatively, mRNA can be isolated from a cell and used to produce cDNA by reverse transcription or other means.

15 The nucleic acids described herein are used in the methods of the present invention for production of proteins or polypeptides, through incorporation into cells, tissues, or organisms. In one embodiment, DNA containing all or part of the coding sequence for a 12q23-qter polypeptide, or DNA which hybridizes to DNA having the sequence of any one of SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687, or a
20 fragment thereof, is incorporated into a vector for expression of the encoded polypeptide in suitable host cells. The encoded amino acid sequence consisting of a 12q23-qter polypeptide, or its functional equivalent is capable of normal activity, such as antigenic or intracellular activity.

25 The invention also concerns the use of the nucleotide sequence of the nucleic acids of this invention to identify DNA probes for 12q23-qter genes, PCR primers to amplify 12q23-qter genes, nucleotide polymorphisms in 12q23-qter genes, and regulatory elements of 12q23-qter genes.

30 The nucleic acids of the present invention find use as primers and templates for the recombinant production of disorder-associated peptides or polypeptides, for chromosome and gene mapping, to provide antisense sequences, for tissue distribution studies, to locate and obtain full length

genes, to identify and obtain homologous sequences (wild-type and mutants), and in diagnostic applications. The primers of this invention may comprise all or a portion of the nucleotide sequence of any one of SEQ ID NO:1 to SEQ ID NO:92, SEQ ID NO:156 to SEQ ID NO:4687, and the
 5 sequences set forth in Tables 8, 9, 11A, and 11B, or a complementary sequence thereof.

Probes may also be used for the detection of 12q23-qter-related sequences, and should preferably contain at least 50%, preferably at least 80%, identity to a 12q23-qter polynucleotide, or a complementary sequence,
 10 or fragments thereof. The probes of this invention may be DNA or RNA, the probes may comprise all or a portion of the nucleotide sequence of any one of SEQ ID NO:1 to SEQ ID NO:92, SEQ ID NO:156 to SEQ ID NO:4687, and the sequences set forth in Tables 8, 9, 11A, and 11B, or a complementary sequence thereof, and may include promoter, enhancer
 15 elements, and introns of the naturally occurring 12q23-qter polynucleotide.

The probes and primers based on the 12q23-qter gene sequences disclosed herein are used to identify homologous 12q23-qter gene sequences and proteins in other species. These 12q23-qter gene sequences and proteins are used in the diagnostic/prognostic, therapeutic
 20 and drug-screening methods described herein for the species from which they have been isolated.

VECTORS AND HOST CELLS

The nucleic acids described herein are used in the methods of the present invention for production of proteins or polypeptides, through
 25 incorporation into cells, tissues, or organisms. In one embodiment, DNA containing all or part of the coding sequence for a chromosome 12q23-qter polypeptide, or DNA which hybridizes to DNA having the sequence SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO: 156 to SEQ ID NO:4687, is incorporated into a vector for expression of the encoded polypeptide in
 30 suitable host cells. The encoded polypeptides consisting of chromosome 12q23-qter genes, or their functional equivalents are capable of normal

activity, such as Gene 702, a metalloprotease-like gene involved in inflammatory responses including tissue destruction and repair. A large number of vectors, including bacterial, yeast, and mammalian vectors, have been described for replication and/or expression in various host cells or cell-free systems, and may be used for gene therapy as well as for simple cloning or protein expression.

In one aspect, an expression vectors comprises a nucleic acid encoding a 12q23-qter polypeptide or peptide, as described herein, operably linked to at least one regulatory sequence. Regulatory sequences are known in the art and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements (see D.V. Goeddel, 1990, *Methods Enzymol.* 185:3-7). Enhancer and other expression control sequences are described in *Enhancers and Eukaryotic Gene Expression*, 1983, Cold Spring Harbor Press, Cold Spring Harbor, NY. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type of polypeptide to be expressed.

Several regulatory elements (e.g., promoters) have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Such regulatory regions, methods of isolation, manner of manipulation, etc. are known in the art. Non-limiting examples of bacterial promoters include the β -lactamase (penicillinase) promoter; lactose promoter; tryptophan (trp) promoter; araBAD (arabinose) operon promoter; lambda-derived P₁ promoter and N gene ribosome binding site; and the hybrid tac promoter derived from sequences of the trp and lac UV5 promoters. Non-limiting examples of yeast promoters include the 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, galactokinase (GAL1) promoter, galactose epimerase promoter, and alcohol dehydrogenase (ADH1) promoter. Suitable promoters for mammalian cells include, without limitation, viral

promoters, such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Preferred replication and inheritance systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, CEN ARS, 2 μ m ARS and the like. While expression
5 vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

To obtain expression in eukaryotic cells, terminator sequences, polyadenylation sequences, and enhancer sequences that modulate gene expression may be required. Sequences that cause amplification of the
10 gene may also be desirable. These sequences are well known in the art. Furthermore, sequences that facilitate secretion of the recombinant product from cells, including, but not limited to, bacteria, yeast, and animal cells, such as secretory signal sequences and/or preprotein or proprotein sequences, may also be included. Such sequences are well described in
15 the art.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells that express the inserts. Typical selection genes
20 encode proteins that 1) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; 2) complement auxotrophic deficiencies, or 3) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli. Markers may be an inducible or non-inducible gene and will generally allow
25 for positive selection. Non-limiting examples of markers include the ampicillin resistance marker (i.e., beta-lactamase), tetracycline resistance marker, neomycin/kanamycin resistance marker (i.e., neomycin phosphotransferase), dihydrofolate reductase, glutamine synthetase, and the like. The choice of the proper selectable marker will depend on the host
30 cell, and appropriate markers for different hosts as understood by those of skill in the art.

Suitable expression vectors for use with the present invention include, but are not limited to, pUC, pBluescript (Stratagene), pET (Novagen, Inc., Madison, WI), and pREP (Invitrogen) plasmids. Vectors can contain one or more replication and inheritance systems for cloning or
 5 expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids. Ligation of the coding sequences to
 10 transcriptional regulatory elements (e.g., promoters, enhancers, and/or insulators) and/or to other amino acid encoding sequences can be carried out using established methods.

Suitable cell-free expression systems for use with the present invention include, without limitation, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *E. coli* S30 extract, and
 15 coupled transcription/translation systems (Promega Corp., Madison, WI). These systems allow the expression of recombinant polypeptides or peptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing protein-coding regions and appropriate promoter elements.

20 Non-limiting examples of suitable host cells include bacteria, archaea, insect, fungi (e.g., yeast), plant, and animal cells (e.g., mammalian, especially human). Of particular interest are *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines.
 25 Techniques for the propagation of mammalian cells in culture are well-known (see, Jakoby and Pastan (eds), 1979, *Cell Culture. Methods in Enzymology*, volume 58, Academic Press, Inc., Harcourt Brace Jovanovich, NY). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although it will
 30 be appreciated by the skilled practitioner that other cell lines may be used,

e.g., to provide higher expression desirable glycosylation patterns, or other features.

Host cells can be transformed, transfected, or infected as appropriate by any suitable method including electroporation, calcium chloride-, lithium chloride-, lithium acetate/polyethylene glycol-, calcium phosphate-, DEAE-dextran-, liposome-mediated DNA uptake, spheroplasting, injection, microinjection, microprojectile bombardment, phage infection, viral infection, or other established methods. Alternatively, vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988, *FEBS Letts.* 241:119). The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

The nucleic acids of the invention may be isolated directly from cells. Alternatively, the polymerase chain reaction (PCR) method can be used to produce the nucleic acids of the invention, using either RNA (e.g., mRNA) or DNA (e.g., genomic DNA) as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression.

Using the information provided in SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687, one skilled in the art will be able to clone and sequence all representative nucleic acids of interest, including nucleic acids encoding complete protein-coding sequences. It is to be understood that non-protein-coding sequences contained within SEQ ID NO:156 to SEQ ID NO:693 and SEQ ID NO:694 to SEQ ID NO:979 are also within the scope of the invention. Such sequences include, without limitation, sequences important for replication, recombination, transcription, and translation. Non-limiting examples include promoters and regulatory binding sites involved in regulation of gene expression, and 5'- and 3'-

untranslated sequences (e.g., ribosome-binding sites) that form part of mRNA molecules.

The nucleic acids of this invention can be produced in large quantities by replication in a suitable host cell. Natural or synthetic nucleic acid fragments, comprising at least ten contiguous bases coding for a desired peptide or polypeptide can be incorporated into recombinant nucleic acid constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the nucleic acid constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cells, cell lines, tissues, or organisms. The purification of nucleic acids produced by the methods of the present invention is described, for example, in Sambrook et al., 1989; F.M. Ausubel et al., 1992, *Current Protocols in Molecular Biology*, J. Wiley and Sons, New York, NY.

The nucleic acids of the present invention can also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage et al., 1981, *Tetra. Letts.* 22:1859-1862, or the triester method according to Matteucci et al., 1981, *J. Am. Chem. Soc.*, 103:3185, and can be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strands together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

These nucleic acids can encode full-length variant forms of proteins as well as the wild-type protein. The variant proteins (which could be especially useful for detection and treatment of disorders) will have the variant amino acid sequences encoded by the polymorphisms described in Table 10, when said polymorphisms are read so as to be in-frame with the full-length coding sequence of which it is a component.

Large quantities of the nucleic acids and proteins of the present invention may be prepared by expressing the 12q23-qter nucleic acids or portions thereof in vectors or other expression vectors in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic
5 hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. For example, insect cell systems (i.e.,
10 lepidopteran host cells and baculovirus expression vectors) are particularly suited for large-scale protein production.

Host cells carrying an expression vector (i.e., transformants or clones) are selected using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule,
15 preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells comprising the nucleic acids of the
20 present invention will be useful not only for the production of the nucleic acids and proteins of the present invention, but also, for example, in studying the characteristics of 12q23-qter proteins. Cells and animals that carry a 12q23-qter gene can be used as model systems to study and test for substances that have potential as therapeutic agents. The cells are typically
25 cultured mesenchymal stem cells. These may be isolated from individuals with a somatic or germline 12q23-qter gene. Alternatively, the cell line can be engineered to carry a 12q23-qter gene, as described above. After a test substance is applied to the cells, the transformed phenotype of the cell is determined. Any trait of transformed cells can be assessed, including
30 respiratory diseases including asthma, atopy, and response to application of putative therapeutic agents.

ANTISENSE NUCLEIC ACIDS

A further embodiment of the invention is antisense nucleic acids or oligonucleotides which are complementary, in whole or in part, to a target molecule comprising a sense strand, and can hybridize with the target molecule. The target can be DNA, or its RNA counterpart (i.e., wherein T residues of the DNA are U residues in the RNA counterpart). When introduced into a cell, antisense nucleic acids or oligonucleotides can inhibit the expression of the gene encoded by the sense strand or the mRNA transcribed from the sense strand. Antisense nucleic acids can be produced by standard techniques. See, for example, Shewmaker, et al., U.S. Patent No. 5,107,065.

In a particular embodiment, an antisense nucleic acid or oligonucleotide is wholly or partially complementary to and can hybridize with a target nucleic acid (either DNA or RNA), wherein the target nucleic acid can hybridize to a nucleic acid having the sequence of the complement of the strands in SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687. For example, an antisense nucleic acid or oligonucleotide can be complementary to a target nucleic acid having the sequence shown as the strand of the open reading frames SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687 or nucleic acids encoding functional equivalents of chromosome 12q23-qter genes, or to a portion of these nucleic acids sufficient to allow hybridization. A portion, for example a sequence of 16 nucleotides, could be sufficient to inhibit expression of the protein. Or, an antisense nucleic acid or oligonucleotide, complementary to 5' or 3' untranslated regions, or overlapping the translation initiation codons (5' untranslated and translated regions), of chromosome 12q23-qter genes, or genes encoding a functional equivalent can also be effective. In another embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize with a target nucleic acid that encodes a chromosome 12q23-qter polypeptide.

In addition to the antisense nucleic acids of the invention, oligonucleotides can be constructed which will bind to duplex nucleic acids either in the genes or the DNA:RNA complexes of transcription, to form stable triple helix-containing or triplex nucleic acids to inhibit transcription and/or expression of a gene encoding a chromosome 12q23-qter gene, or their functional equivalents (Frank-Kamenetskii, M.D. and Mirkin, S.M., 1995, *Ann. Rev. Biochem.* 64:65-95). Such oligonucleotides of the invention are constructed using the base-pairing rules of triple helix formation and the nucleotide sequences of the genes or mRNAs for chromosome 12q23-qter genes.

In preferred embodiments, at least one of the phosphodiester bonds of an antisense oligonucleotide has been substituted with a structure that functions to enhance the ability of the compositions to penetrate into the region of cells where the RNA whose activity is to be modulated is located. It is preferred that such substitutions comprise phosphorothioate bonds, methyl phosphonate bonds, or short chain alkyl or cycloalkyl structures. In accordance with other preferred embodiments, the phosphodiester bonds are substituted with structures which are, at once, substantially non-ionic and non-chiral, or with structures which are chiral and enantiomerically specific. Persons of ordinary skill in the art will be able to select other linkages for use in the practice of the invention.

Oligonucleotides may also include species that include at least some modified base forms. Thus, purines and pyrimidines other than those normally found in nature may be so employed. Similarly, modifications on the furanosyl portions of the nucleotide subunits may also be effected, as long as the essential tenets of this invention are adhered to. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides. Some non-limiting examples of modifications at the 2' position of sugar moieties which are useful in the present invention include OH, SH, SCH₃, F, OCH₃, OCN, O(CH₂)_n NH₂ and O(CH₂)_n CH₃, where n is from 1 to about 10. Such oligonucleotides are functionally interchangeable with natural

oligonucleotides or synthesized oligonucleotides, which have one or more differences from the natural structure. All such analogs are comprehended by this invention so long as they function effectively to hybridize with a 12q23-qter nucleic acid to inhibit the function thereof.

5 The oligonucleotides in accordance with this invention preferably comprise from about 3 to about 50 subunits. It is more preferred that such oligonucleotides and analogs comprise from about 8 to about 25 subunits and still more preferred to have from about 12 to about 20 subunits. As defined herein, a "subunit" is a base and sugar combination suitably bound
10 to adjacent subunits through phosphodiester or other bonds.

Antisense nucleic acids or oligonucleotides can be produced by standard techniques (see, e.g., Shewmaker et al., U.S. Patent No. 5,107,065. The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of
15 solid phase synthesis. Equipment for such synthesis is available from several vendors, including PE Applied Biosystems (Foster City, CA). Any other means for such synthesis may also be employed, however, the actual synthesis of the oligonucleotides is well within the abilities of the practitioner. It is also well known to prepare other oligonucleotide such as
20 phosphorothioates and alkylated derivatives.

The oligonucleotides of this invention are designed to be hybridizable with 12q23-qter RNA (e.g., mRNA) or DNA. For example, an oligonucleotide (e.g., DNA oligonucleotide) that hybridizes to 12q23-qter mRNA can be used to target the mRNA for RnaseH digestion. Alternatively,
25 an oligonucleotide that hybridizes to the translation initiation site of 12q23-qter mRNA can be used to prevent translation of the mRNA. In another approach, oligonucleotides that bind to the double-stranded DNA of 12q23-qter can be administered. Such oligonucleotides can form a triplex construct and inhibit the transcription of the DNA encoding 12q23-qter polypeptides.
30 Triple helix pairing prevents the double helix from opening sufficiently to allow the binding of polymerases, transcription factors, or regulatory

molecules. Recent therapeutic advances using triplex DNA have been described (see, e.g., J.E. Gee et al., 1994, *Molecular and Immunologic Approaches*, Futura Publishing Co., Mt. Kisco, NY).

As non-limiting examples, antisense oligonucleotides may be
5 targeted to hybridize to the following regions: mRNA cap region; translation initiation site; translational termination site; transcription initiation site; transcription termination site; polyadenylation signal; 3' untranslated region; 5' untranslated region; 5' coding region; mid coding region; and 3' coding region. Preferably, the complementary oligonucleotide is designed to
10 hybridize to the most unique 5' sequence of a 12q23-qter gene, including any of about 15-35 nucleotides spanning the 5' coding sequence. Appropriate oligonucleotides can be designed using OLIGO software (Molecular Biology Insights, Inc., Cascade, CO; <http://www.oligo.net>).

In accordance with the present invention, an antisense
15 oligonucleotide can be synthesized, formulated as a pharmaceutical composition, and administered to a subject. The synthesis and utilization of antisense and triplex oligonucleotides have been previously described (e.g., H. Simon et al., 1999, *Antisense Nucleic Acid Drug Dev.* 9:527-31; F.X. Barre et al., 2000, *Proc. Natl. Acad. Sci. USA* 97:3084-3088; R. Elez et al.,
20 2000, *Biochem. Biophys. Res. Commun.* 269:352-6; E.R. Sauter et al., 2000, *Clin. Cancer Res.* 6:654-60). Alternatively, expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well
25 known to those skilled in the art can be used to construct recombinant vectors which will express nucleic acid sequence that is complementary to the nucleic acid sequence encoding a 12q23-qter polypeptide. These techniques are described both in Sambrook et al., 1989 and in Ausubel et al., 1992. For example, 12q23-qter expression can be inhibited by
30 transforming a cell or tissue with an expression vector that expresses high levels of untranslatable 12q23-qter sense or antisense sequences. Even in

the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and even longer if appropriate replication elements included in the

5 vector system.

Various assays may be used to test the ability of antisense oligonucleotides to inhibit 12q23-qter gene expression. For example, 12q23-qter mRNA levels can be assessed Northern blot analysis (Sambrook et al., 1989; Ausubel et al., 1992; J.C. Alwine et al. 1977, *Proc. Natl. Acad. Sci. USA* 74:5350-5354; I.M. Bird, 1998, *Methods Mol. Biol.* 105:325-36),

10 quantitative or semi-quantitative RT-PCR analysis (see, e.g., W.M. Freeman et al., 1999, *Biotechniques* 26:112-122; Ren et al., 1998, *Mol. Brain Res.* 59:256-63; J.M. Cale et al., 1998, *Methods Mol. Biol.* 105:351-71), or *in situ* hybridization (reviewed by A.K. Raap, 1998, *Mutat. Res.* 400:287-298).

15 Alternatively, 12q23-qter polypeptide levels can be measured, e.g., by western blot analysis, indirect immunofluorescence, immunoprecipitation techniques (see, e.g., J.M. Walker, 1998, *Protein Protocols on CD-ROM*, Humana Press, Totowa, NJ).

POLYPEPTIDES

20 The invention also relates to 12q23-qter proteins or polypeptides encoded by the nucleic acids described herein, e.g., SEQ ID NO:93 to SEQ ID NO:155, or portions or variants thereof. The proteins and polypeptides of this invention can be isolated and/or recombinant. In a preferred embodiment, the proteins or portions thereof have at least one function

25 characteristic of a chromosome 12q23-qter protein or polypeptide. For example, Gene 702, a metalloprotease-like gene, the product of which is involved in inflammatory responses including, but not limited to tissue destruction and repair. These proteins are referred to as analogs, and the genes encoding them include, for example, naturally occurring chromosome

30 12q23-qter genes, variants (e.g., mutants) encoding those proteins and/or portions thereof. Such protein or polypeptide variants include mutants

differing by the addition, deletion or substitution of one or more amino acid residues, or modified polypeptides in which one or more residues are modified (e.g., by phosphorylation, sulfation, acylation, etc.), and mutants comprising one or more modified residues. The variant can have

5 "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More infrequently, a variant can have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Guidance in

10 determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be determined using computer programs well known in the art, for example, DNASTAR software (DNASTAR, Inc., Madison, WI).

As non-limiting examples, conservative substitutions in a 12q23-qter amino acid sequence can be made in accordance with the following table:

15

Original Residue	Conservative Substitution(s)	Original Residue	Conservative Substitution(s)
Ala	Ser	Leu	Ile, Val
Arg	Lys	Lys	Arg, Gln, Glu
Asn	Gln, His	Met	Leu, Ile
Asp	Glu	Phe	Met, Leu, Tyr
Cys	Ser	Ser	Thr
Gln	Asn	Thr	Ser
Glu	Asp	Trp	Tyr
Gly	Pro	Tyr	Trp, Phe
His	Asn, Gln	Val	Ile, Leu
Ile	Leu, Val		

Substantial changes in function or immunogenicity can be made by selecting substitutions that are less conservative than those shown in the table, above. For example, non-conservative substitutions can be made

which more significantly affect the structure of the polypeptide in the area of the alteration, for example, the alpha-helical, or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which generally are expected to produce the greatest changes in the polypeptide's properties are those where 1) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; 2) a cysteine or proline is substituted for (or by) any other residue; 3) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or 4) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) a residue that does not have a side chain, e.g., glycine.

In one embodiment, the percent amino acid sequence identity between a chromosome 12q23-qter polypeptide such as SEQ ID NO:93 to SEQ ID NO:155, and functional equivalents thereof is at least 50%. In a preferred embodiment, the percent amino acid sequence identity between such a chromosome 12q23-qter polypeptide and its functional equivalents is at least 65%. More preferably, the percent amino acid sequence identity between a chromosome 12q23-qter polypeptide and its functional equivalents is at least 75%, still more preferably, at least 80%, and even more preferably, at least 90%.

Percent sequence identity can be calculated using computer programs or direct sequence comparison. Preferred computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package, FASTA, BLASTP, and TBLASTN (see, e.g., D.W. Mount, 2001, *Bioinformatics: Sequence and Genome Analysis*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The BLASTP and TBLASTN programs are publicly available from NCBI and other sources. The well-known Smith Waterman algorithm may also be used to determine identity.

Exemplary parameters for amino acid sequence comparison include the following: 1) algorithm from Needleman and Wunsch, 1970, *J Mol. Biol.* 48:443-453; 2) BLOSSUM62 comparison matrix from Hentikoff and Hentikoff, 1992, *Proc. Natl. Acad. Sci. USA* 89:10915-10919; 3) gap penalty = 12; and 4) gap length penalty =4. A program useful with these parameters is publicly available as the "gap" program (Genetics Computer Group, Madison, WI). The aforementioned parameters are the default parameters for polypeptide comparisons (with no penalty for end gaps).

Alternatively, polypeptide sequence identity can be calculated using the following equation: % identity = (the number of identical residues) / (alignment length in amino acid residues) * 100. For this calculation, alignment length includes internal gaps but does not include terminal gaps.

In accordance with the present invention, polypeptide sequences may be identical to the sequence of any one of SEQ ID NO:93 to SEQ ID NO:155, or may include up to a certain integer number of amino acid alterations. Polypeptide alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion. Alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence.

In specific embodiments, a polypeptide variant may be encoded by a 12q23-qter nucleic acid comprising a SNP and/or an alternate splice variant. For example, a polypeptide variant may be encoded by a 12q23-qter alternate splice variant comprising a nucleotide sequence of any one of SEQ ID NO:1 to SEQ ID NO:5; SEQ ID NO:17 to SEQ ID NO:18; SEQ ID NO:36 to SEQ ID NO:37; SEQ ID NO:43 to SEQ ID NO:44; SEQ ID NO:80 to SEQ ID NO:81, or any of the alternate splice sequences set forth in Table 4. In addition, a polypeptide variant may be encoded by a nucleic acid containing one or more 12q23-qter SNPs as set forth in Table 10; Figures 7A-7H;

Figures 9A-9F; Figures 27A-27K; and Figures 28A-28C. Specific examples of amino acid changes encoded by 12q23-qter SNPs are provided in Table 10, and are described in detail hereinbelow.

The invention also relates to isolated, synthesized and/or recombinant portions or fragments of a 12q23-qter protein or polypeptide as described herein. Polypeptide fragments (i.e., peptides) can be made which have full or partial function on their own, or which when mixed together (though fully, partially, or nonfunctional alone), spontaneously assemble with one or more other polypeptides to reconstitute a functional protein having at least one functional characteristic of a 12q23-qter protein of this invention. In addition, 12q23-qter polypeptide fragments may comprise, for example, one or more domains of the 12q23-qter polypeptide, disclosed herein. In particular, a Gene 454 polypeptide may comprise one or more transmembrane, extracellular, or intracellular domains; a Gene 561 polypeptide may comprise a SH3 domain and/or one or more fibronectin type III repeats; and a Gene 757 polypeptide may comprise a cysteine rich domain, a Ser/Thr-XXX-Val motif, and/or one or more transmembrane repeats (see below).

Polypeptides according to the invention can comprise at least 5 contiguous amino acid residues; preferably the polypeptides comprise at least 12 contiguous residues; more preferably the polypeptides comprise at least 20 contiguous residues; and yet more preferably the polypeptides comprise at least 30 contiguous residues. Nucleic acids comprising protein-coding sequences can be used to direct the expression of asthma-associated polypeptides in intact cells or in cell-free translation systems. The coding sequence can be tailored, if desired, for more efficient expression in a given host organism, and can be used to synthesize oligonucleotides encoding the desired amino acid sequences. The resulting oligonucleotides can be inserted into an appropriate vector and expressed in a compatible host organism or translation system.

The polypeptides of the present invention, including function-conservative variants, may be isolated from wild-type or mutant cells (e.g., human cells or cell lines), from heterologous organisms or cells (e.g., bacteria, yeast, insect, plant, and mammalian cells), or from cell-free translation systems (e.g., wheat germ, microsomal membrane, or bacterial extracts) in which a protein-coding sequence has been introduced and expressed. Furthermore, the polypeptides may be part of recombinant fusion proteins. The polypeptides can also, advantageously, be made by synthetic chemistry. Polypeptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis.

Methods for polypeptide purification are well-known in the art, including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. For some purposes, it is preferable to produce the polypeptide in a recombinant system in which the protein contains an additional sequence (e.g., epitope or protein) tag that facilitates purification. Non-limiting examples of epitope tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS) (SEQ ID NO:), GLU-GLU, and DYKDDDDK (SEQ ID NO:) (FLAG®) epitope tags. Non-limiting examples of protein tags include glutathione-S-transferase (GST), green fluorescent protein (GFP), and maltose binding protein (MBP).

In one approach, the coding sequence of a polypeptide or peptide can be cloned into a vector that creates a fusion with a sequence tag of interest. Suitable vectors include, without limitation, pRSET (Invitrogen Corp., San Diego, CA), pGEX (Amersham-Pharmacia Biotech, Inc., Piscataway, NJ), pEGFP (CLONTECH Laboratories, Inc., Palo Alto, CA), and pMAL™ (New England BioLabs (NEB), Inc., Beverly, MA) plasmids. Following expression, the epitope, or protein tagged polypeptide or peptide can be purified from a crude lysate of the translation system or host cell by

chromatography on an appropriate solid-phase matrix. In some cases, it may be preferable to remove the epitope or protein tag (i.e., via protease cleavage) following purification. As an alternative approach, antibodies produced against a disorder-associated protein or against peptides derived therefrom can be used as purification reagents. Other purification methods are also possible.

The present invention also encompasses modifications of 12q23-qter polypeptides. The isolated polypeptides may be modified by, for example, phosphorylation, sulfation, acylation, or other protein modifications. They may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotopes and fluorescent compounds, as described in detail herein.

Both the naturally occurring and recombinant forms of the polypeptides of the invention can advantageously be used to screen compounds for binding activity. Many methods of screening for binding activity are known by those skilled in the art and may be used to practice the invention. Several methods of automated assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period of time. Such high-throughput screening methods are particularly preferred. The use of high-throughput screening assays to test for inhibitors is greatly facilitated by the availability of large amounts of purified polypeptides, as provided by the invention. The polypeptides of the invention also find use as therapeutic agents as well as antigenic components to prepare antibodies.

The polypeptides of this invention find use as immunogenic components useful as antigens for preparing antibodies by standard methods. It is well known in the art that immunogenic epitopes generally contain at least 5 contiguous amino acid residues (Ohno et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:2945). Therefore, the immunogenic components of this invention will typically comprise at least 5 contiguous amino acid residues of the sequence of the complete polypeptide chains. Preferably,

they will contain at least 7, and most preferably at least 10 contiguous amino acid residues or more to ensure that they will be immunogenic. Whether a given component is immunogenic can readily be determined by routine experimentation. Such immunogenic components can be produced by proteolytic cleavage of larger polypeptides or by chemical synthesis or recombinant technology and are thus not limited by proteolytic cleavage sites. The present invention thus encompasses antibodies that specifically recognize asthma-associated immunogenic components.

STRUCTURAL STUDIES

A purified 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155), or portions or complexes thereof, can be analyzed by well-established methods (e.g., X-ray crystallography, NMR, CD, etc.) to determine the three-dimensional structure of the molecule. The three-dimensional structure, in turn, can be used to model intermolecular interactions. Exemplary methods for crystallization and X-ray crystallography are found in P.G. Jones, 1981, *Chemistry in Britain*, 17:222-225; C. Jones et al. (eds), *Crystallographic Methods and Protocols*, Humana Press, Totowa, NJ; A. McPherson, 1982, *Preparation and Analysis of Protein Crystals*, John Wiley & Sons, New York, NY; T.L. Blundell and L.N. Johnson, 1976, *Protein Crystallography*, Academic Press, Inc., New York, NY; A. Holden and P. Singer, 1960, *Crystals and Crystal Growing*, Anchor Books-Doubleday, New York, NY; R.A. Laudise, 1970, *The Growth of Single Crystals*, Solid State Physical Electronics Series, N. Holonyak, Jr., (ed), Prentice-Hall, Inc.; G.H. Stout and L.H. Jensen, 1989, *X-ray Structure Determination: A Practical Guide*, 2nd edition, John Wiley & Sons, New York, NY; *Fundamentals of Analytical Chemistry*, 3rd. edition, Saunders Golden Sunburst Series, Holt, Rinehart and Winston, Philadelphia, PA, 1976; P.D. Boyle of the Department of Chemistry of North Carolina State University at <http://laue.chem.ncsu.edu/web/GrowXtal.html>; M.B. Berry, 1995, *Protein Crystalization: Theory and Practice, Structure and Dynamics*

of *E. coli* Adenylate Kinase, Doctoral Thesis, Rice University, Houston TX;
www.bioc.rice.edu/~berry/papers/crystalization/crystalization.html.

For X-ray diffraction studies, single crystals can be grown to suitable size. Preferably, a crystal has a size of 0.2 to 0.4 mm in at least two of the three dimensions. Crystals can be formed in a solution comprising a 12q23-qter polypeptide (e.g., 1.5-200 mg/ml) and reagents that reduce the solubility to conditions close to spontaneous precipitation. Factors that affect the formation of polypeptide crystals include: 1) purity; 2) substrates or co-factors; 3) pH; 4) temperature; 5) polypeptide concentration; and 6) characteristics of the precipitant. Preferably, the 12q23-qter polypeptides are pure, i.e., free from contaminating components (at least 95% pure), and free from denatured 12q23-qter polypeptides. In particular, polypeptides can be purified by FPLC and HPLC techniques to assure homogeneity (see, Lin et al., 1992, *J. Crystal. Growth*. 122:242-245). Optionally, 12q23-qter polypeptide substrates or co-factors can be added to stabilize the quaternary structure of the protein and promote lattice packing.

Suitable precipitants for crystallization include, but are not limited to, salts (e.g., ammonium sulphate, potassium phosphate); polymers (e.g., polyethylene glycol (PEG) 6000); alcohols (e.g., ethanol); polyalcohols (e.g., 1-methyl-2,4 pentane diol (MPD)); organic solvents; sulfonic dyes; and deionized water. The ability of a salt to precipitate polypeptides can be generally described by the Hofmeister series: $\text{PO}_4^{3-} > \text{HPO}_4^{2-} = \text{SO}_4^{2-} > \text{citrate} > \text{CH}_3\text{CO}_2^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{ClO}_4^- > \text{SCN}^-$; and $\text{NH}_4^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$. Non-limiting examples of salt precipitants are shown below (see Berry, 1995).

Precipitant	Maximum concentration
$(\text{NH}_4^+/\text{Na}^+/\text{Li}^+)_2$ or $\text{Mg}_2+\text{SO}_4^{2-}$	4.0 / 1.5 / 2.1 / 2.5 M
$\text{NH}_4^+/\text{Na}^+/\text{K}^+ \text{PO}_4^{3-}$	3.0 / 4.0 / 4.0 M
$\text{NH}_4^+/\text{K}^+/\text{Na}^+/\text{Li}^+$ citrate	~1.8 M
$\text{NH}_4^+/\text{K}^+/\text{Na}^+/\text{Li}^+$ acetate	~3.0 M
$\text{NH}_4^+/\text{K}^+/\text{Na}^+/\text{Li}^+$ Cl^-	5.2 / 9.8 / 4.2 / 5.4 M

$\text{NH}_4^+\text{NO}_3^-$	$\sim 8.0 \text{ M}$
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High molecular weight polymers useful as precipitating agents include polyethylene glycol (PEG), dextran, polyvinyl alcohol, and polyvinyl pyrrolidone (A. Polson et al., 1964, *Biochem. Biophys. Acta.* 82:463-475). In general, polyethylene glycol (PEG) is the most effective for forming crystals. PEG compounds with molecular weights less than 1000 can be used at concentrations above 40% v/v. PEGs with molecular weights above 1000 can be used at concentration 5-50% w/v. Typically, PEG solutions are mixed with $\sim 0.1 \%$ sodium azide to prevent bacterial growth.

Typically, crystallization requires the addition of buffers and a specific salt content to maintain the proper pH and ionic strength for a protein's stability. Suitable additives include, but are not limited to sodium chloride (e.g., 50-500 mM as additive to PEG and MPD; 0.15-2 M as additive to PEG); potassium chloride (e.g., 0.05-2 M); lithium chloride (e.g., 0.05-2 M); sodium fluoride (e.g., 20-300 mM); ammonium sulfate (e.g., 20-300 mM); lithium sulfate (e.g., 0.05-2 M); sodium or ammonium thiocyanate (e.g., 50-500 mM); MPD (e.g., 0.5-50%); 1,6 hexane diol (e.g., 0.5-10%); 1,2,3 heptane triol (e.g., 0.5-15%); and benzamidine (e.g., 0.5-15%).

Detergents may be used to maintain protein solubility and prevent aggregation. Suitable detergents include, but are not limited to non-ionic detergents such as sugar derivatives, oligoethyleneglycol derivatives, dimethylamine-N-oxides, cholate derivatives, N-octyl hydroxyalkylsulphoxides, sulphobetains, and lipid-like detergents. Sugar-derived detergents include alkyl glucopyranosides (e.g., C8-GP, C9-GP), alkyl thio-glucopyranosides (e.g., C8-tGP), alkyl maltopyranosides (e.g., C10-M, C12-M; CYMAL-3, CYMAL-5, CYMAL-6), alkyl thio-maltopyranosides, alkyl galactopyranosides, alkyl sucroses (e.g., N-octanoylsucrose), and glucamides (e.g., HECAMEG, C-HEGA-10; MEGA-8). Oligoethyleneglycol-derived detergents include alkyl polyoxyethylenes (e.g., C8-E5, C8-En; C12-E8; C12-E9) and phenyl polyoxyethylenes (e.g.,

Triton X-100). Dimethylamine-N-oxide detergents include, e.g., C10-DAO; DDAO; LDAO. Cholate-derived detergents include, e.g., Deoxy-Big CHAP, digitonin. Lipid-like detergents include phosphocholine compounds. Suitable detergents further include zwitter-ionic detergents (e.g.,
 5 ZWITTERGENT 3-10; ZWITTERGENT 3-12); and ionic detergents (e.g., SDS).

Crystallization of macromolecules has been performed at temperatures ranging from 60°C to less than 0°C. However, most molecules can be crystallized at 4°C or 22°C. Lower temperatures promote
 10 stabilization of polypeptides and inhibit bacterial growth. In general, polypeptides are more soluble in salt solutions at lower temperatures (e.g., 4°C), but less soluble in PEG and MPD solutions at lower temperatures. To allow crystallization at 4°C or 22°C, the precipitant or protein concentration can be increased or decreased as required. Heating, melting, and cooling of
 15 crystals or aggregates can be used to enlarge crystals. In addition, crystallization at both 4°C and 22°C can be assessed (A. McPherson, 1992, *J. Cryst. Growth.* 122:161-167; C.W. Carter, Jr. and C.W. Carter, 1979, *J. Biol. Chem.* 254:12219-12223; T. Bergfors, 1993, *Crystallization Lab Manual*).

20 A crystallization protocol can be adapted to a particular polypeptide or peptide. In particular, the physical and chemical properties of the polypeptide can be considered (e.g., aggregation, stability, adherence to membranes or tubing, internal disulfide linkages, surface cysteines, chelating ions, etc.). For initial experiments, the standard set of
 25 crystallization reagents can be used (Hampton Research, Laguna Niguel, CA). In addition, the CRYSTOOL program can provide guidance in determining optimal crystallization conditions (Brent Segelke, 1995, Efficiency analysis of sampling protocols used in protein crystallization screening and crystal structure from two novel crystal forms of PLA2, Ph.D.
 30 Thesis, University of California, San Diego; <http://www.>

ccp14.ac.uk/ccp/web-mirrors/llnlrupp/crystool/crystool.htm).
crystallization conditions are shown below (see Berry, 1995).

Exemplary

Major Precipitant	Additive	Concentration of Major Precipitant	Concentration of Additive
(NH ₄) ₂ SO ₄	PEG 400-2000, MPD, ethanol, or methanol	2.0-4.0 M	6%-0.5%
Na citrate	PEG 400-2000, MPD, ethanol, or methanol	1.4-1.8 M	6%-0.5%
PEG 1000-20000	(NH ₄) ₂ SO ₄ , NaCl, or Na formate	40-50%	0.2-0.6 M

- 5 Robots can be used for automatic screening and optimization of crystallization conditions. For example, the IMPAX and Oryx systems can be used (Douglas Instruments, Ltd., East Garston, United Kingdom). The CRYSTOOL program (Segelke, *supra*) can be integrated with the robotics programming. In addition, the Xact program can be used to construct, maintain, and record the results of various crystallization experiments (see, e.g., D.E. Brodersen et al., 1999, *J. Appl. Cryst.* 32: 1012-1016; G.R. Andersen and J. Nyborg, 1996, *J. Appl. Cryst.* 29:236-240). The Xact program supports multiple users and organizes the results of crystallization experiments into hierarchies. Advantageously, Xact is compatible with both CRYSTOOL and Microsoft® Excel programs.

- Four methods are commonly employed to crystallize macromolecules: vapor diffusion, free interface diffusion, batch, and dialysis. The vapor diffusion technique is typically performed by formulating a 1:1 mixture of a solution comprising the polypeptide of interest and a solution containing the precipitant at the final concentration that is to be achieved after vapor equilibration. The drop containing the 1:1 mixture of protein and precipitant is then suspended and sealed over the well solution, which contains the precipitant at the target concentration, as either a hanging or sitting drop. Vapor diffusion can be used to screen a large number of crystallization conditions or when small amounts of polypeptide

are available. For screening, drop sizes of 1 to 2 μl can be used. Once preliminary crystallization conditions have been determined, drop sizes such as 10 μl can be used. Notably, results from hanging drops may be improved with agarose gels (see K. Provost and M.-C. Robert, 1991, *J. Cryst. Growth* 110:258-264). Free interface diffusion is performed by layering of a low density solution onto one of higher density, usually in the form of concentrated protein onto concentrated salt. Since the solute to be crystallized must be concentrated, this method typically requires relatively large amounts of protein. However, the method can be adapted to work with small amounts of protein. In a representative experiment, 2 to 5 μl of sample is pipetted into one end of a 20 μl microcapillary pipet. Next, 2 to 5 μl of precipitant is pipetted into the capillary without introducing an air bubble, and the ends of the pipet are sealed. With sufficient amounts of protein, this method can be used to obtain relatively large crystals (see, e.g., S.M. Althoff et al., 1988, *J. Mol. Biol.* 199:665-666).

The batch technique is performed by mixing concentrated polypeptide with concentrated precipitant to produce a final concentration that is supersaturated for the solute macromolecule. Notably, this method can employ relatively large amounts of solution (e.g., milliliter quantities), and can produce large crystals. For that reason, the batch technique is not recommended for screening initial crystallization conditions.

The dialysis technique is performed by diffusing precipitant molecules through a semipermeable membrane to slowly increase the concentration of the solute inside the membrane. Dialysis tubing can be used to dialyze milliliter quantities of sample, whereas dialysis buttons can be used to dialyze microliter quantities (e.g., 7-200 μl). Dialysis buttons may be constructed out of glass, perspex, or Teflon™ (see, e.g., Cambridge Repetition Engineers Ltd., Greens Road, Cambridge CB4 3EQ, UK; Hampton Research). Using this method, the precipitating solution can be varied by moving the entire dialysis button or sack into a different solution. In this way, polypeptides can be "reused" until the correct conditions for

crystallization are found (see, e.g., C.W. Carter, Jr. et al., 1988, *J. Cryst. Growth*. 90:60-73). However, this method is not recommended for precipitants comprising concentrated PEG solutions.

Various strategies have been designed to screen crystallization conditions, including 1) pI screening; 2) grid screening; 3) factorials; 4) solubility assays; 5) perturbation; and 6) sparse matrices. In accordance with the pI screening method, the pI of a polypeptide is presumed to be its crystallization point. Screening at the pI can be performed by dialysis against low concentrations of buffer (less than 20 mM) at the appropriate pH, or by use of conventional precipitants.

The grid screening method can be performed on two-dimensional matrices. Typically, the precipitant concentration is plotted against pH. The optimal conditions can be determined for each axis, and then combined. At that point, additional factors can be tested (e.g., temperature, additives). This method works best with fast-forming crystals, and can be readily automated (see M.J. Cox and P.C. Weber, 1988, *J. Cryst. Growth*. 90:318-324). Grid screens are commercially available for popular precipitants such as ammonium sulphate, PEG 6000, MPD, PEG/LiCl, and NaCl (see, e.g., Hamilton Research).

The incomplete factorial method can be performed by 1) selecting a set of ~20 conditions; 2) randomly assigning combinations of these conditions; 3) grading the success of the results of each experiment using an objective scale; and 4) statistically evaluating the effects of each of the conditions on crystal formation (see, e.g., C.W. Carter, Jr. et al., 1988, *J. Cryst. Growth*. 90:60-73). In particular, conditions such as pH, temperature, precipitating agent, and cations can be tested. Dialysis buttons are preferably used with this method. Typically, optimal conditions/combinations can be determined within 35 tests. Similar approaches, such as "footprinting" conditions, may also be employed (see, e.g., E.A. Stura et al., 1991, *J. Cryst. Growth*. 110:1-2).

The perturbation approach can be performed by altering crystallization conditions by introducing a series of additives designed to test the effects of altering the structure of bulk solvent and the solvent dielectric on crystal formation (see, e.g., Whitaker et al., 1995, *Biochem.* 34:8221-8226). Additives for increasing the solvent dielectric include, but are not limited to, NaCl, KCl, or LiCl (e.g., 200 mM); Na formate (e.g., 200 mM); Na₂HPO₄ or K₂HPO₄ (e.g., 200 mM); urea, trichloroacetate, guanidium HCl, or KSCN (e.g., 20-50 mM). A non-limiting list of additives for decreasing the solvent dielectric include methanol, ethanol, isopropanol, or tert-butanol (e.g., 1-5%); MPD (e.g., 1%); PEG 400, PEG 600, or PEG 1000 (e.g., 1-4%); PEG MME (monomethylether) 550, PEG MME 750, PEG MME 2000 (e.g., 1-4%).

As an alternative to the above-screening methods, the sparse matrix approach can be used (see, e.g., J. Jancarik and S.-H.J. Kim, 1991, *Appl. Cryst.* 24:409-411; A. McPherson, 1992, *J. Cryst. Growth.* 122:161-167; B. Cudney et al., 1994, *Acta. Cryst.* D50:414-423). Sparse matrix screens are commercially available (see, e.g., Hampton Research; Molecular Dimensions, Inc., Apopka, FL; Emerald Biostructures, Inc., Lemont, IL). Notably, data from Hampton Research sparse matrix screens can be stored and analyzed using ASPRUN software (Douglas Instruments).

Exemplary conditions for an initial screen are shown below (see Berry, 1995).

TABLE 1A: CRYSTALLIZATION CONDITIONS

Tray 1:

PEG 8000 (wells 1-6)						Ammonium sulfate (wells 7-12)					
1	2	3	4	5	6	7	8	9	10	11	12
20%	20%	20%	35%	35%	35%	2.0 M	2.0 M	2.0 M	2.5 M	2.5 M	2.5 M
pH 5.0	pH 7.0	pH 8.6	pH 5.0	pH 7.0	pH 8.6	pH 5.0	pH 7.0	pH 8.8	pH 5.0	pH 7.0	pH 8.8
MPD (wells 13-16)				Na Citrate (wells 17-20)				Na/K Phosphate (wells 21-24)			
13	14	15	16	17	18	19	20	21	22	23	24
30%	30%	50%	50%	1.3 M	1.3 M	1.5 M	1.5 M	2.0 M	2.0 M	2.5 M	2.5 M
pH 5.8	pH 7.6	pH 5.8	pH 7.6	pH 5.8	pH 7.5	pH 5.8	pH 7.5	pH 6.0	pH 7.4	pH 6.0	pH 7.4

Tray 2:

PEG 2000 MME/0.2 M Ammon. sulfate (wells 25-30)					
25	26	27	28	29	30
25% pH 5.5	25% pH 7.0	25% pH 8.5	40% pH 5.5	40% pH 7.0	40% pH 8.5
Random for wells 31 to 48					

The initial screen can be used with hanging or sitting drops. To conserve the sample, tray 2 can be set up several weeks following tray 1.

- 5 Wells 31-48 of tray 2 can comprise a random set of solutions. Alternatively, solutions can be formulated using sparse methods. Preferably, test solutions cover a broad range of precipitants, additives, and pH (especially pH 5.0-9.0).

- 10 Seeding can be used to trigger nucleation and crystal growth (Stura and Wilson, 1990, *J. Cryst. Growth*. 110:270-282; C. Thaller et al., 1981, *J. Mol. Biol.* 147:465-469; A. McPherson and P. Schlichta, 1988, *J. Cryst. Growth*. 90:47-50). In general, seeding can be performed by transferring crystal seeds into a polypeptide solution to allow polypeptide molecules to deposit on the surface of the seeds and produce crystals. Two seeding
- 15 methods can be used: microseeding and macroseeding. For microseeding, a crystal can be ground into tiny pieces and transferred into the protein solution. Alternatively, seeds can be transferred by adding 1-2 μ l of the seed solution directly to the equilibrated protein solution. In another
- 20 approach, seeds can be transferred by dipping a hair in the seed solution and then streaking the hair across the surface of the drop (streak seeding; see Stura and Wilson, *supra*). For macroseeding, an intact crystal can be transferred into the protein solution (see, e.g., C. Thaller et al., 1981, *J. Mol. Biol.* 147:465-469). Preferably, the surface of the crystal seed is washed to regenerate the growing surface prior to being transferred. Optimally, the
- 25 protein solution for crystallization is close to saturation and the crystal seed is not completely dissolved upon transfer.

ANTIBODIES

Another aspect of the invention pertains to antibodies directed to 12q23-qter polypeptides, or portions or variants thereof. The invention provides polyclonal and monoclonal antibodies that bind 12q23-qter polypeptides or peptides. The antibodies may be elicited in an animal host (e.g., rabbit, goat, mouse, or other non-human mammal) by immunization with disorder-associated immunogenic components. Antibodies may also be elicited by *in vitro* immunization (sensitization) of immune cells. The immunogenic components used to elicit the production of antibodies may be isolated from cells or chemically synthesized. The antibodies may also be produced in recombinant systems programmed with appropriate antibody-encoding DNA. Alternatively, the antibodies may be constructed by biochemical reconstitution of purified heavy and light chains. The antibodies include hybrid antibodies, chimeric antibodies, and univalent antibodies. Also included are Fab fragments, including Fab¹ and Fab(ab)² fragments of antibodies.

In accordance with the present invention, antibodies are directed to a 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155), or variants, or portions thereof. For example, antibodies can be produced to bind to a 12q23-qter polypeptide encoded by an alternate splice variant comprising a nucleotide sequence of any one of SEQ ID NO:1 to SEQ ID NO:5; SEQ ID NO:17 to SEQ ID NO:18; SEQ ID NO:36 to SEQ ID NO:37; SEQ ID NO:43 to SEQ ID NO:44; SEQ ID NO:80 to SEQ ID NO:81; or any of the alternate splice sequences set forth in Table 4. As another example, antibodies can be produced to bind to a 12q23-qter polypeptide variant encoded by a nucleic acid containing one or more 12q23-qter SNPs as set forth in Table 10; Figures 7A-7H; Figures 9A-9F; Figures 27A-27K; and Figures 28A-28C. Such antibodies can be used as diagnostic and/or therapeutic reagents.

An isolated 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155), or variant, or portion thereof, can be used as an immunogen to

generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 12q23-qter polypeptide can be used or, alternatively, the invention provides antigenic peptide portions of 12q23-qter for use as immunogens. The antigenic peptide of 12q23-qter comprises at least 5 contiguous amino acid residues of the amino acid sequence shown in any one of SEQ ID NO:93 to SEQ ID NO:155, or a variant thereof, and encompasses an epitope of a 12q23-qter polypeptide such that an antibody raised against the peptide forms a specific immune complex with A 12q23-qter amino acid sequence.

- 10 An appropriate immunogenic preparation can contain, for example, recombinantly produced 12q23-qter polypeptide or a chemically synthesized 12q23-qter polypeptide, or portions thereof. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. A number of adjuvants are known and used by those skilled in the art. Non-limiting examples of suitable adjuvants include incomplete Freund's adjuvant, mineral gels such as alum, aluminum phosphate, aluminum hydroxide, aluminum silica, and surface-active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Further examples of adjuvants include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3 hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. A particularly useful adjuvant comprises 5% (wt/vol) squalene, 2.5% Pluronic L121 polymer and 0.2% polysorbate in phosphate buffered saline (Kwak et al., 1992, *New Eng. J. Med.* **327**:1209-1215). Preferred adjuvants include complete BCG, Detox, (RIBI, Immunochem Research Inc.), ISCOMS, and aluminum hydroxide adjuvant

(Superphos, Biosector). The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide.

Polyclonal antibodies to 12q23-qter polypeptides can be prepared as described above by immunizing a suitable subject with a 12q23-qter immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 12q23-qter polypeptide or peptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction.

At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique (see Kohler and Milstein, 1975, *Nature* 256:495-497; Brown et al., 1981, *J. Immunol.* 127:539-46; Brown et al., 1980, *J. Biol. Chem.* 255:4980-83; Yeh et al., 1976, *PNAS* 76:2927-31; and Yeh et al., 1982, *Int. J. Cancer* 29:269-75), the human B cell hybridoma technique (Kozbor et al., 1983, *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques.

The technology for producing hybridomas is well-known (see generally R. H. Kenneth, 1980, *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, NY; E.A. Lerner, 1981, *Yale J. Biol. Med.*, 54:387-402; M.L. Geffer et al., 1977, *Somatic Cell Genet.* 3:231-36). In general, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a 12q23-qter immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds 12q23-qter polypeptides or peptides.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an monoclonal antibody to a 12q23-qter polypeptide (see, e.g., G. Galfre et al., 1977, *Nature* 266:55052; Gefter et al., 1977; Lerner, 1981; Kenneth, 1980).

5 Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of
10 the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin, and thymidine (HAT medium). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-
15 x63-Ag8.653, or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC (American Type Culture Collection, Manassas, VA). Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol (PEG). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused
20 and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind 12q23-qter polypeptides or peptides, e.g., using a standard ELISA assay.

25 Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the corresponding 12q23-qter polypeptide to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for
30 generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-

9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612).

- Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; Dower et al. PCT International Publication No. WO 91/17271; Winter et al. PCT International Publication WO 92/20791; Markland et al. PCT International Publication No. WO 92/15679; Breitling et al. PCT International Publication WO 93/01288; McCafferty et al. PCT International Publication No. WO 92/01047; Garrard et al. PCT International Publication No. WO 92/09690; Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al., 1991, *Bio/Technology* 9:1370-1372; Hay et al., 1992, *Hum. Antibod. Hybridomas* 3:81-85; Huse et al., 1989, *Science* 246:1275-1281; Griffiths et al., 1993, *EMBO J* 12:725-734; Hawkins et al., 1992, *J. Mol. Biol.* 226:889-896; Clarkson et al., 1991, *Nature* 352:624-628; Gram et al., 1992, *PNAS* 89:3576-3580; Garrad et al., 1991, *Bio/Technology* 9:1373-1377; Hoogenboom et al., 1991, *Nuc. Acid Res.* 19:4133-4137; Barbas et al., 1991, *PNAS* 88:7978-7982; and McCafferty et al., 1990, *Nature* 348:552-55.
- Additionally, recombinant antibodies to a 12q23-qter polypeptide, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al. European Patent Application 125,023; Better et al., 1988, *Science* 240:1041-1043; Liu et al., 1987, *PNAS* 84:3439-3443; Liu et al., 1987, *J.*

Immunol. 139:3521-3526; Sun et al., 1987, *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al., 1985, *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl. Cancer Inst.* 80:1553-1559; S.L. Morrison, 1985, *Science* 229:1202-1207; Oi et al., 1986, *BioTechniques* 4:214; Winter
 5 U.S. Pat. No. 5,225,539; Jones et al., 1986, *Nature* 321:552-525; Verhoeyan et al., 1988, *Science* 239:1534; and Bcidler et al., 1988, *J. Immunol.* 141:4053-4060.

An antibody against a 12q23-qter polypeptide (e.g., monoclonal antibody) can be used to isolate the corresponding polypeptide by standard
 10 techniques, such as affinity chromatography or immunoprecipitation. For example, antibodies can facilitate the purification of a natural 12q23-qter polypeptide from cells and of a recombinantly produced 12q23-qter polypeptide or peptide expressed in host cells. In addition, an antibody that binds to a 12q23-qter polypeptide can be used to detect the corresponding
 15 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Such antibodies can also be used diagnostically to monitor 12q23-qter protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen as described in detail herein. In
 20 addition, antibodies to a 12q23-qter polypeptide can be used as therapeutics for the treatment of diseases related to abnormal 12q23-qter gene expression or function, e.g., asthma.

LIGANDS

The 12q23-qter polypeptides (e.g., SEQ ID NO:93 to SEQ ID
 25 NO:155), polynucleotides (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687), variants, or fragments or portions thereof, can be used to screen for ligands (e.g., agonists, antagonists, or inhibitors) that modulate the levels or activity of the 12q23-qter polypeptide. In addition, these 12q23-qter molecules can be used to identify endogenous ligands that
 30 bind to 12q23-qter polypeptides or polynucleotides in the cell. In one aspect of the present invention, the full-length 12q23-qter polypeptide (e.g., SEQ ID

NO:93 to SEQ ID NO:155) is used to identify ligands. Alternatively, variants or portions of a 12q23-qter polypeptide are used. Such portions may comprise, for example, one or more domains of the 12q23-qter polypeptide (e.g., transmembrane, intracellular, extracellular, SH3, fibronectin III repeat, cysteine-rich, and Ser/Thr-XXX-Val domains) disclosed herein. Of particular interest are screening assays that identify agents that have relatively low levels of toxicity in human cells. A wide variety of assays may be used for this purpose, including *in vitro* protein-protein binding assays, electrophoretic mobility shift assays, immunoassays, and the like.

Ligands that bind to the 12q23-qter polypeptides or polynucleotides of the invention are potentially useful in diagnostic applications and/or pharmaceutical compositions, as described in detail herein. Ligands may encompass numerous chemical classes, though typically they are organic molecules, e.g., small molecules. Preferably, small molecules have a molecular weight of less than 5000 daltons, more preferably, small molecules have a molecular weight of more than 50 and less than 2,500 daltons. Such molecules can comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. Useful molecules often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Such molecules can also comprise biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs, or combinations thereof.

Ligands may include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, e.g., Lam et al., 1991, *Nature* 354:82-84; Houghten et al., 1991, *Nature* 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (e.g., members of random and partially degenerate, directed

phosphopeptide libraries, see, e.g., Songyang et al, 1993, *Cell* 72:767-778);

3) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab')₂, Fab expression library fragments, and epitope-binding fragments of antibodies);

5 and 4) small organic and inorganic molecules.

Test agents useful for identifying 12q23-qter ligands can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Synthetic compound libraries are commercially available from, for example, Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex
10 (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich Chemical Company, Inc. (Milwaukee, WI). Natural compound libraries comprising bacterial, fungal, plant or animal extracts are available from, for example, Pan Laboratories (Bothell, WA). In addition, numerous means are available
15 for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides.

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be readily produced. Methods for the synthesis of molecular libraries are readily available (see, e.g., DeWitt et al.,
20 1993, *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al., 1994, *J. Med. Chem.* 37:2678; Cho et al., 1993, *Science* 261:1303; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al., 1994, *J. Med. Chem.* 37:1233). In addition, natural or
25 synthetic compound libraries and compounds can be readily modified through conventional chemical, physical and biochemical means (see, e.g., Blondelle et al., 1996, *Trends in Biotech.* 14:60), and may be used to produce combinatorial libraries. In another approach, previously identified pharmacological agents can be subjected to directed or random chemical
30 modifications, such as acylation, alkylation, esterification, amidification, and the analogs can be screened for 12q23-qter gene-modulating activity.

Numerous methods for producing combinatorial libraries are known in the art, including those involving biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and
 5 synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds (K. S. Lam, 1997, *Anticancer Drug Des.* 12:145).

- 10 Non-limiting examples of small molecules, small molecule libraries, combinatorial libraries, and screening methods are described in B. Seligmann, 1995, "Synthesis, Screening, Identification of Positive Compounds and Optimization of Leads from Combinatorial Libraries: Validation of Success" p. 69-70. *Symposium: Exploiting Molecular Diversity: Small Molecule Libraries for Drug Discovery*, La Jolla, CA, Jan. 23-25, 1995
 15 (conference summary available from Wendy Warr & Associates, 6 Berwick Court, Cheshire, UK CW4 7HZ); E. Martin et al., 1995, *J. Med. Chem.* 38:1431-1436; E. Martin et al., 1995, "Measuring diversity: Experimental design of combinatorial libraries for drug discovery" Abstract, ACS Meeting, Anaheim, CA, COMP 32; and E. Martin, 1995, "Measuring Chemical Diversity: Random Screening or Rationale Library Design" p. 27-30, *Symposium: Exploiting Molecular Diversity: Small Molecule Libraries for Drug Discovery*, La Jolla, Calif. Jan. 23-25, 1995 (conference summary available from Wendy Warr & Associates, 6 Berwick Court, Cheshire, UK
 20 CW4 7HZ).
 25

Libraries may be screened in solution (e.g., Houghten, 1992, *Biotechniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria or spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull et al., 1992, *Proc. Natl. Acad. Sci. USA*
 30 89:1865-1869), or on phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla et al., 1990, *Proc. Natl. Acad.*

Sci. USA 97:6378-6382; Felici, 1991, *J. Mol. Biol.* 222:301-310; Ladner, *supra*).

Where the screening assay is a binding assay, a 12q23-qter polypeptide, polynucleotide, analog, or fragment thereof, may be joined to a
5 label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemiluminescers, enzymes, specific binding molecules, particles, e.g., magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin, etc. For the specific binding members,
10 the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc., that are used to facilitate optimal protein-protein binding and/or reduce
15 non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The components are added in any order that produces the requisite binding. Incubations are performed at any temperature that facilitates optimal activity, typically between 4° and 40°C.
20 Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Normally, between 0.1 and 1 hr will be sufficient. In general, a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to these concentrations. Typically, one of these concentrations
25 serves as a negative control, i.e., at zero concentration or below the level of detection.

To perform cell-free ligand screening assays, it may be desirable to immobilize either a 12q23-qter polypeptide, polynucleotide, or fragment to a surface to facilitate identification of ligands that bind to these molecules, as
30 well as to accommodate automation of the assay. For example, a fusion protein comprising a 12q23-qter polypeptide and an affinity tag can be

produced. In one embodiment, a glutathione-S-transferase/phosphodiesterase fusion protein comprising a 12q23-qter polypeptide is adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione-derivatized microtiter plates. Cell lysates (e.g., containing ³⁵S-labeled polypeptides) are added to the coated beads under conditions to allow complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the coated beads are washed to remove any unbound polypeptides, and the amount of immobilized radiolabel is determined. Alternatively, the complex is dissociated and the radiolabel present in the supernatant is determined. In another approach, the beads are analyzed by SDS-PAGE to identify the bound polypeptides.

Ligand-binding assays can be used to identify agonist or antagonists that alter the function or levels of a 12q23-qter polypeptide. Such assays are designed to detect the interaction of test agents (e.g., small molecules) with 12q23-qter polypeptides, polynucleotides, analogs, or fragments or portions thereof. Interactions may be detected by direct measurement of binding. Alternatively, interactions may be detected by indirect indicators of binding, such as stabilization/destabilization of protein structure, or activation/inhibition of biological function. Non-limiting examples of useful ligand-binding assays are detailed below.

Ligands that bind to 12q23-qter polypeptides, polynucleotides, analogs, or fragments or portions thereof, can be identified using real-time Bimolecular Interaction Analysis (BIA; Sjolander et al., 1991, *Anal. Chem.* 63:2338-2345; Szabo et al., 1995, *Curr. Opin. Struct. Biol.* 5:699-705). BIA-based technology (e.g., BIAcoreTM; LKB Pharmacia, Sweden) allows study of biospecific interactions in real time, without labeling. In BIA, changes in the optical phenomenon surface plasmon resonance (SPR) is used to determine real-time interactions of biological molecules.

Ligands can also be identified by scintillation proximity assays (SPA, described in U.S. Patent No. 4,568,649). In a modification of this assay that

is currently undergoing development, chaperonins are used to distinguish folded and unfolded proteins. A tagged protein is attached to SPA beads, and test agents are added. The bead is then subjected to mild denaturing conditions (such as, e.g., heat, exposure to SDS, etc.) and a purified labeled
5 chaperonin is added. If a test agent binds to a target, the labeled chaperonin will not bind; conversely, if no test agent binds, the protein will undergo some degree of denaturation and the chaperonin will bind.

Ligands can also be identified using a binding assay based on mitochondrial targeting signals (Hurt et al., 1985, *EMBO J.* 4:2061-2068;
10 Eilers and Schatz, 1986, *Nature* 322:228-231). In a mitochondrial import assay, expression vectors are constructed in which nucleic acids encoding particular target proteins are inserted downstream of sequences encoding mitochondrial import signals. The chimeric proteins are synthesized and tested for their ability to be imported into isolated mitochondria in the
15 absence and presence of test compounds. A test compound that binds to the target protein should inhibit its uptake into isolated mitochondria *in vitro*.

The ligand-binding assay described in Fodor et al., 1991, *Science* 251:767-773, which involves testing the binding affinity of test compounds for a plurality of defined polymers synthesized on a solid substrate, can also
20 be used.

Ligands that bind to 12q23-qter polypeptides or peptides can be identified using two-hybrid assays (see, e.g., U.S. Pat. No. 5,283,317; Zervos et al., 1993, *Cell* 72:223-232; Madura et al., 1993, *J. Biol. Chem.* 268:12046-12054; Bartel et al., 1993, *Biotechniques* 14:920-924; Iwabuchi
25 et al., 1993, *Oncogene* 8:1693-1696; and Brent WO 94/10300). The two-hybrid system relies on the reconstitution of transcription activation activity by association of the DNA-binding and transcription activation domains of a transcriptional activator through protein-protein interaction. The yeast GAL4 transcriptional activator may be used in this way, although other
30 transcription factors have been used and are well known in the art. To carryout the two-hybrid assay, the GAL4 DNA-binding domain, and the

GAL4 transcription activation domain are expressed, separately, as fusions to potential interacting polypeptides.

In one embodiment, the "bait" protein comprises a 12q23-qter polypeptide fused to the GAL4 DNA-binding domain. The "fish" protein
5 comprises, for example, a human cDNA library encoded polypeptide fused to the GAL4 transcription activation domain. If the two, coexpressed fusion proteins interact in the nucleus of a host cell, a reporter gene (e.g., LacZ) is activated to produce a detectable phenotype. The host cells that show two-hybrid interactions can be used to isolate the containing plasmids containing
10 the cDNA library sequences. These plasmids can be analyzed to determine the nucleic acid sequence and predicted polypeptide sequence of the candidate ligand. Alternatively, methods such as the three-hybrid (Licitra et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:12817-12821), and reverse two-hybrid (Vidal et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:10315-10320)
15 systems may be used. Commercially available two-hybrid systems such as the CLONTECH Matchmaker™ systems and protocols (CLONTECH Laboratories, Inc., Palo Alto, CA) may be also be used (see also, A.R. Mendelsohn et al., 1994, *Curr. Op. Biotech.* 5:482; E.M. Phizicky et al., 1995, *Microbiological Rev.* 59:94; M. Yang et al., 1995, *Nucleic Acids Res.*
20 23:1152; S. Fields et al., 1994, *Trends Genet.* 10:286; and U.S. Patent No. 6,283,173 and 5,468,614).

Several methods of automated assays have been developed in recent years so as to permit screening of tens of thousands of test agents in a short period of time. High-throughput screening methods are particularly
25 preferred for use with the present invention. The ligand-binding assays described herein can be adapted for high-throughput screens, or alternative screens may be employed. For example, continuous format high throughput screens (CF-HTS) using at least one porous matrix allows the researcher to test large numbers of test agents for a wide range of biological or
30 biochemical activity (see United States Patent No. 5,976,813 to Beutel et al.). Moreover, CF-HTS can be used to perform multi-step assays.

DIAGNOSTICS

As discussed herein, 12q23-qter genes are associated with various diseases and disorders, including but not limited to, asthma, atopy, obesity, male germ cell tumors, histidinemia, growth retardation with deafness and mental retardation, deficiency of Acyl-CoA dehydrogenase, spinal muscular atrophy, Darier disease, cardiomyopathy, Spinocerebellar ataxia-2, brachydactyly, Mevalonicaciduria, Hyperimmunoglobulinemia D, Noonan syndrome-1, Cardiofaciocutaneous syndrome, spinal muscular atrophy-4, tyrosinemia, phenylketonuria, B-cell non-Hodgkin lymphoma, Ulnar-mammary syndrome, Holt-Oram syndrome, Scapulooperoneal spinal muscular atrophy, alcohol intolerance, MODY, diabetes mellitus, non-insulin-dependent type 2, diabetes mellitus insulin-dependent (See National Center for Biotechnology Information at <http://www.ncbi.nlm.nih.gov/omim/>), and inflammatory bowel disease (B. Wallaert et al., 1995, *J. Exp. Med.* 182:1897-1904). The present invention therefore provides nucleic acids and antibodies that can be useful in diagnosing individuals with disorders associated with aberrant 12q23-qter gene expression and/or mutated 12q23-qter genes. In particular, nucleic acids comprising 12q23-qter SNPs can be used to identify chromosomal abnormalities linked to these diseases. Additionally, antibodies directed against the amino acid variants encoded by the 12q23-qter SNPs can be used to identify disease-associated polypeptides.

Antibody-based diagnostic methods: In a further embodiment of the present invention, antibodies which specifically bind to a 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155) may be used for the diagnosis of conditions or diseases characterized by underexpression or overexpression of the 12q23-qter polynucleotide or polypeptide, or in assays to monitor patients being treated with a 12q23-qter polypeptide, polynucleotide, or antibody, or a 12q23-qter agonist, antagonist, or inhibitor.

The antibodies useful for diagnostic purposes may be prepared in the same manner as those for use in therapeutic methods, described herein.

Antibodies may be raised to a full-length 12q23-qter polypeptide sequence (e.g., SEQ ID NO:93 to SEQ ID NO:155). Alternatively, the antibodies may be raised to portions or variants of the 12q23-qter polypeptide. Such variants include polypeptides encoded by the disclosed 12q23-qter SNPs or alternate splice variants. In one aspect of the invention, antibodies are prepared to bind to a 12q23-qter polypeptide fragment comprising one or more domains of the 12q23-qter polypeptide (e.g., transmembrane, intracellular, extracellular, SH3, fibronectin III repeat, cysteine-rich, and Ser/Thr-XXX-Val domains), as described in detail herein.

Diagnostic assays for a 12q23-qter polypeptide include methods that utilize the antibody and a label to detect the protein in biological samples (e.g., human body fluids, cells, tissues, or extracts of cells or tissues). The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules that are known in the art may be used, several of which are described herein.

The invention provides methods for detecting disease-associated antigenic components in a biological sample, which methods comprise the steps of: 1) contacting a sample suspected to contain a disease-associated antigenic component with an antibody specific for an disease-associated antigen, extracellular or intracellular, under conditions in which an antigen-antibody complex can form between the antibody and disease-associated antigenic components in the sample; and 2) detecting any antigen-antibody complex formed in step (1) using any suitable means known in the art, wherein the detection of a complex indicates the presence of disease-associated antigenic components in the sample. It will be understood that assays that utilize antibodies directed against altered 12q23-qter amino acid sequences (i.e., epitopes encoded by SNPs, modifications, mutations, or variants) are within the scope of the invention.

Many immunoassay formats are known in the art, and the particular format used is determined by the desired application. An immunoassay can

use, for example, a monoclonal antibody directed against a single disease-associated epitope, a combination of monoclonal antibodies directed against different epitopes of a single disease-associated antigenic component, monoclonal antibodies directed towards epitopes of different disease-associated antigens, polyclonal antibodies directed towards the same disease-associated antigen, or polyclonal antibodies directed towards different disease-associated antigens. Protocols can also, for example, use solid supports, or may involve immunoprecipitation.

In accordance with the present invention, "competitive" (U.S. Pat. Nos. 3,654,090 and 3,850,752), "sandwich" (U.S. Pat. No. 4,016,043), and "double antibody," or "DASP" assays may be used. Several procedures for measuring the amount of a 12q23-qter polypeptide in a sample (e.g., ELISA, RIA, and FACS) are known in the art and provide a basis for diagnosing altered or abnormal levels of 12q23-qter polypeptide expression. Normal or standard values for a 12q23-qter polypeptide expression are established by incubating biological samples taken from normal subjects, preferably human, with antibody to a 12q23-qter polypeptide under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods; photometric means are preferred. Levels of the 12q23-qter polypeptide expressed in the subject sample, negative control (normal) sample, and positive control (disease) sample are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

Typically, immunoassays use either a labeled antibody or a labeled antigenic component (i.e., to compete with the antigen in the sample for binding to the antibody). A number of fluorescent materials are known and can be utilized as labels for antibodies or polypeptides. These include, for example, Cy3, Cy5, GFP (e.g., EGFP, DsRed, dEFP, etc. (CLONTECH, Palo Alto, CA)), Alexa, BODIPY, fluorescein (e.g., FluorX, DTAF, and FITC), rhodamine (e.g., TRITC), auramine, Texas Red, AMCA blue, and Lucifer Yellow. Antibodies or polypeptides can also be labeled with a radioactive

element or with an enzyme. Preferred isotopes include ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re .

Preferred enzymes include peroxidase, β -glucuronidase, β -D-glucosidase, β -D-galactosidase, urease, glucose oxidase plus peroxidase, and alkaline phosphatase (see, e.g., U.S. Pat. Nos. 3,654,090; 3,850,752 and 4,016,043). Enzymes can be conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde, and the like. Enzyme labels can be detected visually, or measured by calorimetric, spectrophotometric, fluorospectrophotometric, amperometric, or gasometric techniques. Other labeling systems, such as avidin/biotin, Tyramide Signal Amplification (TSA™), are known in the art, and are commercially available (see, e.g., ABC kit, Vector Laboratories, Inc., Burlingame, CA; NEN® Life Science Products, Inc., Boston, MA).

Kits suitable for antibody-based diagnostic applications typically include one or more of the following components:

- (1) Antibodies: The antibodies may be pre-labeled; alternatively, the antibody may be unlabeled and the ingredients for labeling may be included in the kit in separate containers, or a secondary, labeled antibody is provided; and
- (2) Reaction components: The kit may also contain other suitably packaged reagents and materials needed for the particular immunoassay protocol, including solid-phase matrices, if applicable, and standards.

The kits referred to above may include instructions for conducting the test. Furthermore, in preferred embodiments, the diagnostic kits are adaptable to high-throughput and/or automated operation.

Nucleic-acid-based diagnostic methods: The invention provides methods for detecting altered levels or sequences of 12q23-qter nucleic acids (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687) in a sample, such as in a biological sample, comprising the steps of: 1) contacting a sample suspected to contain a disease-associated nucleic acid with one or more disease-associated nucleic acid probes under

conditions in which hybrids can form between any of the probes and disease-associated nucleic acid in the sample; and 2) detecting any hybrids formed in step (1) using any suitable means known in the art, wherein the detection of hybrids indicates the presence of the disease-associated nucleic acid in the sample. Exemplary methods are described in Examples 9 and 10, herein below. To detect disease-associated nucleic acids present in low levels in biological samples, it may be necessary to amplify the disease-associated sequences or the hybridization signal as part of the diagnostic assay. Techniques for amplification are known to those of skill in the art.

The presence of a 12q23-qter polynucleotide sequences can be detected by DNA-DNA or DNA-RNA hybridization, or by amplification using probes or primers comprising at least a portion of a 12q23-qter polynucleotide, or a sequence complementary thereto. In particular, nucleic acid amplification-based assays can use 12q23-qter oligonucleotides or oligomers to detect transformants containing 12q23-qter DNA or RNA. Preferably, 12q23-qter nucleic acids useful as probes in diagnostic methods include oligonucleotides at least 15 contiguous nucleotides in length, more preferably at least 20 contiguous nucleotides in length, and most preferably at least 25-55 contiguous nucleotides in length, that hybridize specifically with 12q23-qter nucleic acids. As non-limiting examples, probes or primers useful for diagnostics may comprise any of the 12q23-qter DNA nucleotide sequences shown in Tables 8, 9, 11A, and 11B.

Several methods can be used to produce specific probes for 12q23-qter polynucleotides. For example, labeled probes can be produced by oligo-labeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, 12q23-qter polynucleotide sequences (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687), or any portions or fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in*

vitro by addition of an appropriate RNA polymerase, such as T7, T3, or SP(6) and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (e.g., from Amersham-Pharmacia; Promega Corp.; and U.S. Biochemical Corp., Cleveland, OH). Suitable reporter molecules or labels which may be used include radionucleotides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

A sample to be analyzed, such as, for example, a tissue sample (e.g., hair or buccal cavity) or body fluid sample (e.g., blood or saliva), may be contacted directly with the nucleic acid probes. Alternatively, the sample may be treated to extract the nucleic acids contained therein. It will be understood that the particular method used to extract DNA will depend on the nature of the biological sample. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques, or, the nucleic acid sample may be immobilized on an appropriate solid matrix without size separation.

Kits suitable for nucleic acid-based diagnostic applications typically include the following components:

(1)Probe DNA: The probe DNA may be prelabeled; alternatively, the probe DNA may be unlabeled and the ingredients for labeling may be included in the kit in separate containers; and

(2)Hybridization reagents: The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, including solid-phase matrices, if applicable, and standards.

In cases where a disease condition is suspected to involve an alteration of a 12q23-qter nucleotide sequence, specific oligonucleotides may be constructed and used to assess the level of disease mRNA in cells affected or other tissue affected by the disease. For example, PCR can be used to test whether a person has a disease-related polymorphism (i.e., mutation). Specific methods of polymorphism identification are described herein, but are not intended to limit the present invention. The detection of

polymorphisms in DNA sequences can be accomplished by a variety of methods including, but not limited to, RFLP detection based on allele-specific restriction-endonuclease cleavage (Kan and Dozy, 1978, *Lancet* ii:910-912), hybridization with allele-specific oligonucleotide probes (Wallace et al., 1978, *Nucl Acids Res.* 6:3543-3557), including immobilized oligonucleotides (Saiki et al., 1969, *Proc. Natl. Acad. Sci. USA* 86:6230-6234) or oligonucleotide arrays (Maskos and Southern, 1993, *Nucl. Acids Res.* 21:2269-2270), allele-specific PCR (Newton et al., 1989, *Nucl. Acids Res.* 17:2503-2516), mismatch-repair detection (MRD) (Faham and Cox, 1995, *Genome Res.* 5:474-482), binding of MutS protein (Wagner et al., 1995, *Nucl. Acids Res.* 23:3944-3948), denaturing-gradient gel electrophoresis (DGGE) (Fisher and Lerman et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:1579-1583), single-strand-conformation-polymorphism detection (Orita et al., 1983, *Genomics* 5:874-879), RNAase cleavage at mismatched base-pairs (Myers et al., 1985, *Science* 230:1242), chemical (Cotton et al., 1988, *Proc. Natl. Acad. Sci. USA* 8:4397-4401) or enzymatic (Youil et al., 1995, *Proc. Natl. Acad. Sci. USA* 92:87-91) cleavage of heteroduplex DNA, methods based on allele specific primer extension (Syvanen et al., 1990, *Genomics* 8:684-692), genetic bit analysis (GBA) (Nikiforov et al., 1994, *Nucl. Acids* 22:4167-4175), the oligonucleotide-ligation assay (OLA) (Landegren et al., 1988, *Science* 241:1077), the allele-specific ligation chain reaction (LCR) (Barrany, 1991, *Proc. Natl. Acad. Sci. USA* 88:189-193), gap-LCR (Abravaya et al., 1995, *Nucl. Acids Res.* 23:675-682), radioactive and/or fluorescent DNA sequencing using standard procedures well known in the art, and peptide nucleic acid (PNA) assays (Orum et al., 1993, *Nucl. Acids Res.* 21:5332-5356).

For PCR analysis, 12q23-qter oligonucleotides may be chemically synthesized, generated enzymatically, or produced from a recombinant source. Oligomers will preferably comprise two nucleotide sequences, one with a sense orientation (5' → 3') and another with an antisense orientation (3' → 5'), employed under optimized conditions for identification of a specific

gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantification of closely related DNA or RNA sequences.

5 In accordance with PCR analysis, two oligonucleotides are synthesized by standard methods or are obtained from a commercial supplier of custom-made oligonucleotides. The length and base composition are determined by standard criteria using the Oligo 4.0 primer Picking program (W. Rychlik, 1992; available from Molecular Biology
10 Insights, Inc., Cascade, CO). One of the oligonucleotides is designed so that it will hybridize only to the disease gene DNA under the PCR conditions used. The other oligonucleotide is designed to hybridize a segment of genomic DNA such that amplification of DNA using these oligonucleotide primers produces a conveniently identified DNA fragment. Samples may be
15 obtained from hair follicles, whole blood, or the buccal cavity. The DNA fragment generated by this procedure is sequenced by standard techniques.

In one particular aspect, 12q23-qter oligonucleotides can be used to perform Genetic Bit Analysis (GBA) of 12q23-qter genes in accordance with published methods (T.T. Nikiforov et al., 1994, *Nucleic Acids Res.*
20 **22(20):4167-75**; T.T. Nikiforov et al., 1994, *PCR Methods Appl.* **3(5):285-91**). In PCR-based GBA, specific fragments of genomic DNA containing the polymorphic site(s) are first amplified by PCR using one unmodified and one phosphorothioate-modified primer. The double-stranded PCR product is rendered single-stranded and then hybridized to immobilized oligonucleotide
25 primer in wells of a multi-well plate. The primer is designed to anneal immediately adjacent to the polymorphic site of interest. The 3' end of the primer is extended using a mixture of individually labeled dideoxynucleoside triphosphates. The label on the extended base is then determined. Preferably, GBA is performed using semi-automated ELISA or biochip
30 formats (see, e.g., S.R. Head et al., 1997, *Nucleic Acids Res.* **25(24):5065-71**; T.T. Nikiforov et al., 1994, *Nucleic Acids Res.* **22(20):4167-75**).

Other amplification techniques besides PCR may be used as alternatives, such as ligation-mediated PCR or techniques involving Q-beta replicase (Cahill et al., 1991, *Clin. Chem.*, 37(9):1482-5). Products of amplification can be detected by agarose gel electrophoresis, quantitative hybridization, or equivalent techniques for nucleic acid detection known to one skilled in the art of molecular biology (Sambrook et al., 1989). Other alterations in the disease gene may be diagnosed by the same type of amplification-detection procedures, by using oligonucleotides designed to contain and specifically identify those alterations.

In accordance with the present invention, 12q23-qter polynucleotides may also be used to detect and quantify levels of 12q23-qter mRNA in biological samples in which altered expression of 12q23-qter polynucleotide may be correlated with disease. These diagnostic assays may be used to distinguish between the absence, presence, increase, and decrease of 12q23-qter mRNA levels, and to monitor regulation of 12q23-qter polynucleotide levels during therapeutic treatment or intervention. For example, 12q23-qter polynucleotide sequences, or fragments, or complementary sequences thereof, can be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or biochip assays utilizing fluids or tissues from patient biopsies to detect the status of, e.g., levels or overexpression of 12q23-qter genes, or to detect altered 12q23-qter gene expression. Such qualitative or quantitative methods are well known in the art (G.H. Keller and M.M. Manak, 1993, *DNA Probes*, 2nd Ed, Macmillan Publishers Ltd., England; D.W. Dieffenbach and G. S. Dveksler, 1995, *PCR Primer: A Laboratory Manual*, Cold Spring Harbor Press, Plainview, NY; B.D. Hames and S.J. Higgins, 1985, *Gene Probes* 1, 2, IRL Press at Oxford University Press, Oxford, England).

Methods suitable for quantifying the expression of 12q23-qter genes include radiolabeling or biotinylating nucleotides, co-amplification of a control nucleic acid, and standard curves onto which the experimental

results are interpolated (P.C. Melby et al., 1993, *J. Immunol. Methods* 159:235-244; and C. Duplaa et al., 1993, *Anal. Biochem.* 212(1):229-36.). The speed of quantifying multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantification.

In accordance with these methods, the specificity of the probe, i.e., whether it is made from a highly specific region (e.g., at least 8 to 10 or 12 or 15 contiguous nucleotides in the 5' regulatory region), or a less specific region (e.g., especially in the 3' coding region), and the stringency of the hybridization or amplification (e.g., high, moderate, or low) will determine whether the probe identifies naturally occurring sequences encoding the 12q23-qter polypeptide, or alleles, SNPs, mutants, or related sequences.

In a particular aspect, a 12q23-qter nucleic acid sequence (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687), or a sequence complementary thereto, or fragment thereof, may be useful in assays that detect 12q23-qter-related diseases such as asthma. A 12q23-qter polynucleotide can be labeled by standard methods, and added to a biological sample from a subject under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample can be washed and the signal is quantified and compared with a standard value. If the amount of signal in the test sample is significantly altered from that of a comparable negative control (normal) sample, the altered levels of a 12q23-qter nucleotide sequence can be correlated with the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular prophylactic or therapeutic regimen in animal studies, in clinical trials, or for an individual patient.

To provide a basis for the diagnosis of a disease associated with altered expression of a 12q23-qter gene, a normal or standard profile for expression is established. This may be accomplished by incubating biological samples taken from normal subjects, either animal or human, with

a sequence complementary to the 12q23-qter polynucleotide, or a fragment thereof, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for the disease. Deviation between standard and subject (patient) values is used to establish the presence of the condition.

Once the disease is diagnosed and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in a normal individual. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to diseases such as asthma, the presence of an abnormal amount of a 12q23-qter transcript in a biological sample (e.g., body fluid, cells, tissues, or cell or tissue extracts) from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier, thereby preventing the development or further progression of the disease.

Microarrays: In another embodiment of the present invention, oligonucleotides, or longer fragments derived from a 12q23-qter polynucleotide sequence described herein may be used as targets in a microarray (e.g., biochip) system. The microarray can be used to monitor the expression level of large numbers of genes simultaneously (to produce a transcript image), and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function,

to understand the genetic basis of a disease, to diagnose disease, and to develop and monitor the activities of therapeutic or prophylactic agents. Preparation and use of microarrays have been described in WO 95/11995 to Chee et al.; D.J. Lockhart et al., 1996, *Nature Biotechnology* 14:1675-1680; 5 M. Schena et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:10614-10619; U.S. Patent No. 6,015,702 to P. Lal et al; J. Worley et al., 2000, *Microarray Biochip Technology*, M. Schena, ed., Biotechniques Book, Natick, MA, pp. 65-86; Y.H. Rogers et al., 1999, *Anal. Biochem.* 266(1):23-30; S.R. Head et al., 1999, *Mol. Cell. Probes.* 13(2):81-7; S.J. Watson et al., 2000, *Biol.* 10 *Psychiatry* 48(12):1147-56.

In one application of the present invention, microarrays containing arrays of 12q23-qter polynucleotide sequences can be used to measure the expression levels of 12q23-qter nucleic acids in an individual. In particular, to diagnose an individual with a 12q23-qter-related condition or disease, a 15 sample from a human or animal (containing nucleic acids, e.g., mRNA) can be used as a probe on a biochip containing an array of 12q23-qter polynucleotides (e.g., DNA) in decreasing concentrations (e.g., 1 ng, 0.1 ng, 0.01 ng, etc.). The test sample can be compared to samples from diseased and normal samples. Biochips can also be used to identify 12q23-qter 20 mutations or polymorphisms in a population, including but not limited to, deletions, insertions, and mismatches. For example, mutations can be identified by: 1) placing 12q23-qter polynucleotides of this invention onto a biochip; 2) taking a test sample (containing, e.g., mRNA) and adding the sample to the biochip; 3) determining if the test samples hybridize to the 25 12q23-qter polynucleotides attached to the chip under various hybridization conditions (see, e.g., V.R. Chechetkin et al., 2000, *J. Biomol. Struct. Dyn.* 18(1):83-101). Alternatively microarray sequencing can be performed (see, e.g., E.P. Diamandis, 2000, *Clin. Chem.* 46(10):1523-5).

Chromosome mapping: In another application of this invention, 30 12q23-qter nucleic acid sequences (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO: 156 to SEQ ID NO:4687), or complementary sequences, or

fragments thereof, can be used as probes to map genomic sequences. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to human artificial chromosome constructions (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial PI constructions, or single chromosome cDNA libraries (see, e.g., C.M. Price, 1993, *Blood Rev.*, 7:127-134; B.J. Trask, 1991, *Trends Genet.* 7:149-154).

In another of its aspects, the invention relates to a diagnostic kit for detecting a 12q23-qter polynucleotide or polypeptide as it relates to a disease or susceptibility to a disease, particularly asthma. Also related is a diagnostic kit that can be used to detect or assess asthma conditions. Such kits comprise one or more of the following:

- (a) a 12q23-qter polynucleotide, preferably the nucleotide sequence of any one of SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687, or a fragment thereof; or
- (b) a nucleotide sequence complementary to that of (a); or
- (c) a 12q23-qter polypeptide, preferably the polypeptide of any one of SEQ ID NO:93 to SEQ ID NO:155, or a fragment thereof; or
- (d) an antibody to a 12q23-qter polypeptide, preferably to the polypeptide of any one of SEQ ID NO:93 to SEQ ID NO:155, or an antibody bindable fragment thereof. It will be appreciated that in any such kits, (a), (b), (c), or (d) may comprise a substantial component and that instructions for use can be included. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

The present invention also includes a test kit for genetic screening that can be utilized to identify mutations in 12q23-qter genes. By identifying patients with mutated 12q23-qter DNA and comparing the mutation to a database that contains known mutations in 12q23-qter and a particular condition or disease, identification and/or confirmation of, a particular condition or disease can be made. Accordingly, such a kit would comprise a PCR-based test that would involve transcribing the patients mRNA with a

specific primer, and amplifying the resulting cDNA using another set of primers. The amplified product would be detectable by gel electrophoresis and could be compared with known standards for 12q23-qter genes. Preferably, this kit would utilize a patient's blood, serum, or saliva sample, and the DNA would be extracted using standard techniques. Primers flanking a known mutation would then be used to amplify a fragment of a 12q23-qter gene. The amplified piece would then be sequenced to determine the presence of a mutation.

Genomic Screening: Polymorphic genetic markers linked to a 12q23-qter gene can be used to predict susceptibility to the diseases genetically linked to that chromosomal region. Similarly, the identification of polymorphic genetic markers within 12q23-qter genes will allow the identification of specific allelic variants that are in linkage disequilibrium with other genetic lesions that affect one of the disease states discussed herein including respiratory disorders, obesity, and inflammatory bowel disease. SSCP (see below) allows the identification of polymorphisms within the genomic and coding region of the disclosed genes.

The present invention provides sequences for primers that can be used identify exons that contain SNPs, as well as sequences for primers that can be used to identify the sequence changes of the SNPs. In particular, Table 10 shows polymorphic genetic markers within the chromosome 12q23-qter genes, which can be used to identify specific allelic variants that are in linkage disequilibrium with other genetic lesions that affect one of the disease states discussed herein, including respiratory disorders, obesity, and inflammatory bowel disease. Such markers can be used in conjunction with SSCP to identify polymorphisms within the genomic and coding region of the disclosed gene. Table 8 shows primers that can be used to identify exons containing SNPs. Table 9 shows primers that can be used to identify the sequence changes of the SNPs.

This information can be used to identify additional SNPs in accordance with the methods disclosed herein. Suitable methods for

genomic screening have also been described by, e.g., Sheffield et al., 1995, *Genet.* 4:1837-1844; LeBlanc-Straceski et al., 1994, *Genomics* 19:341-9; Chen et al., 1995, *Genomics* 25:1-8. In employing these methods, the disclosed reagents can be used to predict the risk for disease (e.g., respiratory disorders, obesity, and inflammatory bowel disease) in a population or individual.

THERAPEUTICS

As discussed herein, 12q23-qter genes are associated with various diseases and disorders, including but not limited to, asthma, atopy, obesity, male germ cell tumors, histidinemia, growth retardation with deafness and mental retardation, deficiency of Acyl-CoA dehydrogenase, spinal muscular atrophy, Darier disease, cardiomyopathy, Spinocerebellar ataxia-2, brachydactyly, Mevalonicaciduria, Hyperimmunoglobulinemia D, Noonan syndrome-1, Cardiofaciocutaneous syndrome, spinal muscular atrophy-4, tyrosinemia, phenylketonuria, B-cell non-Hodgkin lymphoma, Ulnar-mammary syndrome, Holt-Oram syndrome, Scapulooperoneal spinal muscular atrophy, alcohol intolerance, MODY, diabetes mellitus, non-insulin-dependent type 2, diabetes mellitus insulin-dependent (See National Center for Biotechnology Information at <http://www.ncbi.nlm.nih.gov/omim/>), and inflammatory bowel disease (B. Wallaert et al., 1995, *J. Exp. Med.* 182:1897-1904). The present invention therefore provides compositions (e.g., pharmaceutical compositions) comprising 12q23-qter nucleic acids, polypeptides, antibodies, ligands, or variants, portions, or fragments thereof that can be useful in treating individuals with these disorders. Also provided are methods employing 12q23-qter nucleic acids, polypeptides, antibodies, ligands, or variants, portions, or fragments thereof to identify drug candidates that can be used to prevent, treat, or ameliorate such disorders.

Drug screening and design: The present invention provides methods of screening for drugs using a 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155), or portion thereof, in competitive binding assays, according to methods well-known in the art. For example, competitive drug

screening assays can be employed using neutralizing antibodies capable of specifically binding a 12q23-qter polypeptide compete with a test compound for binding to the 12q23-qter polypeptide or fragments thereof.

The present invention further provides methods of rational drug design employing a 12q23-qter polypeptide, antibody, or portion or functional equivalent thereof. The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, or inhibitors). In turn, these analogs can be used to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of the polypeptide *in vivo* (see, e.g., Hodgson, 1991, *Bio/Technology*, 9:19-21). An example of rational drug design is the development of HIV protease inhibitors (Erickson et al., 1990, *Science*, 249:527-533).

In one approach, one first determines the three-dimensional structure of a protein of interest or, for example, of a 12q23-qter polypeptide or ligand complex, by x-ray crystallography, computer modeling, or a combination thereof. Useful information regarding the structure of a polypeptide can also be gained by computer modeling based on the structure of homologous proteins. In addition, 12q23-qter polypeptides (e.g., SEQ ID NO:93 to SEQ ID NO:155), or portions thereof, can be analyzed by an alanine scan (Wells, 1991, *Methods in Enzymol.*, 202:390-411). In this technique, each amino acid residue in a 12q23-qter polypeptide is replaced by alanine, and its effect on the activity of the polypeptide is determined.

In another approach, an antibody specific to a 12q23-qter polypeptide can be isolated, selected by a functional assay, and then analyzed to solve its crystal structure. In principle, this approach can yield a pharmacore upon which subsequent drug design can be based. Alternatively, it is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the

binding site of the anti-ids is predicted to be an analog of the corresponding 12q23-qter polypeptide. The anti-id can then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides can subsequently be used as pharmacores.

- 5 Non-limiting examples of methods and computer tools for drug design are described in R. Cramer et al., 1974, *J. Med. Chem.* 17:533; H. Kubinyi (ed) 1993, *3D QSAR in Drug Design, Theory, Methods, and Applications*, ESCOM, Leiden, Holland; P. Dean (ed) 1995, *Molecular Similarity in Drug Design*, K. Kim "Comparative molecular field analysis (ComFA)" p. 291-324, 10 Chapman & Hill, London, UK; Y. et al., 1993, *J. Comp.-Aid. Mol. Des.* 7:83-102; G. Lauri and P.A. Bartlett, 1994, *J. Comp.-Aid. Mol. Des.* 8:51-66; P.J. Gane and P.M. Dean, 2000, *Curr. Opin. Struct. Biol.* 10(4):401-4; H.O. Kim and M. Kahn, 2000, *Comb. Chem. High Throughput Screen.* 3(3):167-83; G.K. Farber, 1999, *Pharmacol Ther.* 84(3):327-32; and H. van de 15 Waterbeemd (ed) 1996, *Structure-Property Correlations in Drug Research*, Academic Press, San Diego, CA.

In another aspect of the present invention, cells and animals that carry a 12q23-qter gene or an analog thereof can be used as model systems to study and test for substances that have potential as therapeutic 20 agents. After a test agent is administered to animals or applied to the cells, the phenotype of the animals/cells can be determined.

In accordance with these methods, one may design drugs that result in, for example, altered 12q23-qter polypeptide activity or stability. Such drugs may act as inhibitors, agonists, or antagonists of a 12q23-qter 25 polypeptide. By virtue of the availability of cloned 12q23-qter gene sequences, sufficient amounts of the 12q23-qter polypeptide may be produced to perform such analytical studies as x-ray crystallography. In addition, the knowledge of the 12q23-qter polypeptide sequence will guide those employing computer-modeling techniques in place of, or in addition to 30 x-ray crystallography.

Pharmaceutical compositions: The present invention contemplates compositions comprising a 12q23-qter polynucleotide (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687), polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155), antibody, ligand (e.g., agonist, antagonist, or inhibitor), or fragments, variants, or analogs thereof, and a physiologically acceptable carrier, excipient, or diluent as described in detail herein. The present invention further contemplates pharmaceutical compositions useful in practicing the therapeutic methods of this invention. Preferably, a pharmaceutical composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of a 12q23-qter polypeptide, polynucleotide, ligand, antibody, or fragment, portion, or variant thereof, as described herein, as an active ingredient. The preparation of pharmaceutical compositions that contain 12q23-qter molecules as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH-buffering agents, which enhance the effectiveness of the active ingredient.

A 12q23-qter polypeptide, polynucleotide, ligand, antibody, or fragment, portion, or variant thereof can be formulated into the pharmaceutical composition as neutralized physiologically acceptable salt forms. Suitable salts include the acid addition salts (i.e., formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic,

and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

5 The pharmaceutical compositions can be administered systemically by oral or parenteral routes. Non-limiting parenteral routes of administration include subcutaneous, intramuscular, intraperitoneal, intravenous, transdermal, inhalation, intranasal, intra-arterial, intrathecal, enteral, sublingual, or rectal. Intravenous administration, for example, can be
10 performed by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e.,
15 carrier, or vehicle.

In one particular embodiment of the present invention, the disclosed pharmaceutical compositions are administered via mucoactive aerosol therapy (see, e.g., M. Fuloria and B.K. Rubin, 2000, *Respir. Care* 45:868-873; I. Gonda, 2000, *J. Pharm. Sci.* 89:940-945; R. Dhand, 2000, *Curr.*
20 *Opin. Pulm. Med.* 6(1):59-70; B.K. Rubin, 2000, *Respir. Care* 45(6):684-94; S. Suarez and A.J. Hickey, 2000, *Respir. Care* 45(6):652-66).

Pharmaceutical compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be
25 treated, capacity of the subject's immune system to utilize the active ingredient, and degree of modulation of 12q23-qter gene activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are specific for each individual. However, suitable dosages may range from about 0.1 to 20, preferably
30 about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend

on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous
5 infusions sufficient to maintain concentrations of 10 nM to 10 μ M in the blood are contemplated. An exemplary pharmaceutical formulation comprises: 12q23-qter antagonist or inhibitor (5.0 mg/ml); sodium bisulfite USP (3.2 mg/ml); disodium edetate USP (0.1 mg/ml); and water for injection q.s.a.d. (1.0 ml). As used herein, "pg" means picogram, "ng" means
10 nanogram, " μ g" means microgram, "mg" means milligram, " μ l" means microliter, "ml" means milliliter, and "l" means L.

For further guidance in preparing pharmaceutical formulations, see, e.g., Gilman et al. (eds), 1990, *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, 8th ed., Pergamon Press; and
15 *Remington's Pharmaceutical Sciences*, 17th ed., 1990, Mack Publishing Co., Easton, PA; Avis et al. (eds), 1993, *Pharmaceutical Dosage Forms: Parenteral Medications*, Dekker, New York; Lieberman et al. (eds), 1990, *Pharmaceutical Dosage Forms: Disperse Systems*, Dekker, New York.

In yet another aspect of this invention, antibodies that specifically
20 react with a 12q23-qter polypeptide or peptides derived therefrom can be used as therapeutics. In particular, such antibodies can be used to block the activity of a 12q23-qter polypeptide. Antibodies or fragments thereof can be formulated as pharmaceutical compositions and administered to a subject. It is noted that antibody-based therapeutics produced from non-
25 human sources can cause an undesired immune response in human subjects. To minimize this problem, chimeric antibody derivatives can be produced. Chimeric antibodies combine a non-human animal variable region with a human constant region. Chimeric antibodies can be constructed according to methods known in the art (see Morrison et al.,
30 1985, *Proc. Natl. Acad. Sci. USA* 81:6851; Takeda et al., 1985, *Nature* 314:452; U.S. Patent No. 4,816,567 of Cabilly et al.; U.S. Patent No.

4,816,397 of Boss et al.; European Patent Publication EP 171496; EP 0173494; United Kingdom Patent GB 2177096B).

In addition, antibodies can be further "humanized" by any of the techniques known in the art, (e.g., Teng et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:7308-7312; Kozbor et al., 1983, *Immunology Today* 4: 7279; Olsson et al., 1982, *Meth. Enzymol.* 92:3-16; International Patent Application WO92/06193; EP 0239400). Humanized antibodies can also be obtained from commercial sources (e.g., Scotgen Limited, Middlesex, England). Immunotherapy with a humanized antibody may result in increased long-term effectiveness for the treatment of chronic disease situations or situations requiring repeated antibody treatments.

Pharmacogenetics: The 12q23-qter polynucleotides (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687) and polypeptides (e.g., SEQ ID NO:93 to SEQ ID NO:155) of the invention are also useful in pharmacogenetic analysis (i.e., the study of the relationship between an individual's genotype and that individual's response to a therapeutic composition or drug). See, e.g., M. Eichelbaum, 1996, *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985, and M.W. Linder, 1997, *Clin. Chem.* 43(2):254-266. The genotype of the individual can determine the way a therapeutic acts on the body or the way the body metabolizes the therapeutic. Further, the activity of drug metabolizing enzymes affects both the intensity and duration of therapeutic activity. Differences in the activity or metabolism of therapeutics can lead to severe toxicity or therapeutic failure. Accordingly, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenetic studies in determining whether to administer a 12q23-qter polypeptide, polynucleotide, analog, antagonist, inhibitor, or modulator, as well as tailoring the dosage and/or therapeutic or prophylactic treatment regimen.

In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions can be due to a single factor that alters the way the drug act on the body (altered drug action), or a factor that alters

the way the body metabolizes the drug (altered drug metabolism). These conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy which results in
 5 haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why
 10 some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. The gene coding
 15 for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response. This has been
 20 demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme, ultra-rapid metabolizers fail to respond to standard doses. Recent studies have determined that ultra-rapid metabolism is attributable to CYP2D6 gene amplification.

By analogy, genetic polymorphism or mutation may lead to allelic
 25 variants of 12q23-qter genes in the population which have different levels of activity. The 12q23-qter polypeptides or polynucleotides thereby allow a clinician to ascertain a genetic predisposition that can affect treatment modality. In addition, genetic mutation or variants at other genes may potentiate or diminish the activity of 12q23-qter-targeted drugs. Thus, in a
 30 12q23-qter gene-based treatment, a polymorphism or mutation may give rise to individuals that are more or less responsive to treatment.

Accordingly, dosage would necessarily be modified to maximize the therapeutic effect within a given population containing the polymorphism. As an alternative to genotyping, specific polymorphic polypeptides or polynucleotides can be identified.

- 5 To identify genes that modify 12q23-qter-targeted drug response, several pharmacogenetic methods can be used. One pharmacogenomics approach, "genome-wide association", relies primarily on a high-resolution map of the human genome. This high-resolution map shows previously identified gene-related markers (e.g., a "bi-allelic" gene marker map which
- 10 consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). A high-resolution genetic map can then be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect.
- 15 Alternatively, a high-resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In this way, treatment
- 20 regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals (see, e.g., D.R. Pfoest et al., 2000, *Trends Biotechnol.* 18(8):334-8).

- As another example, the "candidate gene approach", can be used.
- 25 According to this method, if a gene that encodes a drug target is known, all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

- As yet another example, a "gene expression profiling approach", can
- 30 be used. This method involves testing the gene expression of an animal treated with a drug (e.g., a 12q23-qter polypeptide, polynucleotide, analog,

or modulator) to determine whether gene pathways related to toxicity have been turned on.

Information obtained from one of the approaches described herein can be used to establish a pharmacogenetic profile, which can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. A pharmacogenetic profile, when applied to dosing or drug selection, can be used to avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 12q23-qter polypeptide, polynucleotide, analog, antagonist, inhibitor, or modulator.

The 12q23-qter polypeptides or polynucleotides of the invention are also useful for monitoring therapeutic effects during clinical trials and other treatment. Thus, the therapeutic effectiveness of an agent that is designed to increase or decrease gene expression, polypeptide levels, or activity can be monitored over the course of treatment using the 12q23-qter compositions or modulators. For example, monitoring can be performed by: 1) obtaining a pre-administration sample from a subject prior to administration of the agent; 2) detecting the level of expression or activity of the protein in the pre-administration sample; 3) obtaining one or more post-administration samples from the subject; 4) detecting the level of expression or activity of the polypeptide in the post-administration samples; 5) comparing the level of expression or activity of the polypeptide in the pre-administration sample with the polypeptide in the post-administration sample or samples; and 6) increasing or decreasing the administration of the agent to the subject accordingly.

Gene Therapy: The 12q23-qter polynucleotides (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687) and polypeptides (e.g., SEQ ID NO:93 to SEQ ID NO:155) of the invention also find use as gene therapy reagents. In recent years, significant technological advances have been made in the area of gene therapy for both genetic and acquired diseases (Kay et al., 1997, *Proc. Natl. Acad. Sci. USA*, 94:12744-

12746). Gene therapy can be defined as the transfer of DNA for therapeutic purposes. Improvement in gene transfer methods has allowed for development of gene therapy protocols for the treatment of diverse types of diseases. Gene therapy has also taken advantage of recent advances in the identification of new therapeutic genes, improvement in both viral and non-viral gene delivery systems, better understanding of gene regulation, and improvement in cell isolation and transplantation. Gene therapy would be carried out according to generally accepted methods as described by, for example, Friedman, 1991, *Therapy for Genetic Diseases*, Friedman, Ed., Oxford University Press, pages 105-121.

Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation, and viral transduction are known in the art, and the choice of method is within the competence of one skilled in the art (Robbins (ed), 1997, *Gene Therapy Protocols*, Human Press, NJ). Cells transformed with a 12q23-qter gene can be used as model systems to study chromosome 12 disorders and to identify drug treatments for the treatment of such disorders.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and non-viral transfer methods. A number of viruses have been used as gene transfer vectors, including polyoma, *i.e.*, SV40 (Madzak et al., 1992, *J. Gen. Virol.*, 73:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, 158:39-6; Berkner et al., 1988, *Bio Techniques*, 6:616-629; Gorziglia et al., 1992, *J. Virol.*, 66:4407-4412; Quantin et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:2581-2584; Rosenfeld et al., 1992, *Cell*, 68:143-155; Wilkinson et al., 1992, *Nucl. Acids Res.*, 20:2233-2239; Stratford-Perricaudet et al., 1990, *Hum. Gene Ther.*, 1:241-256), vaccinia virus (Mackett et al., 1992, *Biotechnology*, 24:495- 499), adeno-associated virus (Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.*, 158:91- 123; Ohi et al.,

1990, *Gene*, 89:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.*, 158:67-90; Johnson et al., 1992, *J. Virol.*, 66:2952-2965; Fink et al., 1992, *Hum. Gene Ther.*, 3:11-19; Breakfield et al., 1987, *Mol. Neurobiol.*, 1:337-371; Fresse et al., 1990, 5 *Biochem. Pharmacol.*, 40:2189-2199), and retroviruses of avian (Brandyopadhyay et al., 1984, *Mol. Cell Biol.*, 4:749-754; Petropoulos et al., 1992, *J. Virol.*, 66:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.*, 158:1-24; Miller et al., 1985, *Mol. Cell Biol.*, 5:431-437; Sorge et al., 1984, *Mol. Cell Biol.*, 4:1730-1737; Mann et al., 1985, *J. Virol.*, 54:401-10 407), and human origin (Page et al., 1990, *J. Virol.*, 64:5370-5276; Buchschalcher et al., 1992, *J. Virol.*, 66:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses.

Non-viral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham et al., 1973, 15 *Virology*, 52:456-467; Pellicer et al., 1980, *Science*, 209:1414-1422), mechanical techniques, for example microinjection (Anderson et al., 1980, *Proc. Natl. Acad. Sci. USA*, 77:5399-5403; Gordon et al., 1980, *Proc. Natl. Acad. Sci. USA*, 77:7380-7384; Brinster et al., 1981, *Cell*, 27:223-231; Constantini et al., 1981, *Nature*, 294:92-94), membrane fusion-mediated 20 transfer via liposomes (Felgner et al., 1987, *Proc. Natl. Acad. Sci. USA*, 84:7413-7417; Wang et al., 1989, *Biochemistry*, 28:9508-9514; Kaneda et al., 1989, *J. Biol. Chem.*, 264:12126-12129; Stewart et al., 1992, *Hum. Gene Ther.*, 3:267-275; Nabel et al., 1990, *Science*, 249:1285-1288; Lim et al., 1992, *Circulation*, 83:2007-2011), and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990, *Science*, 247:1465-1468; Wu et al., 1991, *BioTechniques*, 11:474-485; Zenke et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:3655-3659; Wu et al., 1989, *J. Biol. Chem.*, 264:16985-16987; Wolff et al., 1991, *BioTechniques*, 11:474-485; Wagner et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:4255-4259; Cotten et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:4033-4037; Curiel et al., 1991, *Proc. Natl. Acad. Sci. USA*, 30 88:8850-8854; Curiel et al., 1991, *Hum. Gene Ther.*, 3:147-154).

In one approach, plasmid DNA is complexed with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged. In another approach, liposome/DNA is used to mediate direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is non-specific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992, *Hum. Gene Ther.*, 3:399-410).

Suitable gene transfer vectors possess a promoter sequence, preferably a promoter that is cell-specific and placed upstream of the sequence to be expressed. The vectors may also contain, optionally, one or more expressible marker genes for expression as an indication of successful transfection and expression of the nucleic acid sequences contained in the vector. In addition, vectors can be optimized to minimize undesired immunogenicity and maximize long-term expression of the desired gene product(s) (see Nabe, 1999, *Proc. Natl. Acad. Sci. USA* 96:324-326). Moreover, vectors can be chosen based on cell-type that is targeted for treatment. Notably, gene transfer therapies have been initiated for the treatment of various pulmonary diseases (see, e.g., M.J. Welsh, 1999, *J. Clin. Invest.* 104(9):1165-6; D.L. Ennist, 1999, *Trends Pharmacol. Sci.* 20:260-266; S.M. Albelda et al., 2000, *Ann. Intern. Med.* 132:649-660; E. Alton and C. Kitson C., 2000, *Expert Opin. Investig. Drugs.* 9(7):1523-35).

Illustrative examples of vehicles or vector constructs for transfection or infection of the host cells include replication-defective viral vectors, DNA virus or RNA virus (retrovirus) vectors, such as adenovirus, herpes simplex virus and adeno-associated viral vectors. Adeno-associated virus vectors are single stranded and allow the efficient delivery of multiple copies of nucleic acid to the cell's nucleus. Preferred are adenovirus vectors. The vectors will normally be substantially free of any prokaryotic DNA and may

comprise a number of different functional nucleic acid sequences. An example of such functional sequences may be a DNA region comprising transcriptional and translational initiation and termination regulatory sequences, including promoters (e.g., strong promoters, inducible
5 promoters, and the like) and enhancers which are active in the host cells. Also included as part of the functional sequences is an open reading frame (polynucleotide sequence) encoding a protein of interest. Flanking sequences may also be included for site-directed integration. In some
10 situations, the 5'-flanking sequence will allow homologous recombination, thus changing the nature of the transcriptional initiation region, so as to provide for inducible or non-inducible transcription to increase or decrease the level of transcription, as an example.

In general, the encoded and expressed 12q23-qter polypeptide may be intracellular, i.e., retained in the cytoplasm, nucleus, or in an organelle, or
15 may be secreted by the cell. For secretion, the natural signal sequence present in a 12q23-qter polypeptide may be retained. When the polypeptide or peptide is a fragment of a 12q23-qter protein, a signal sequence may be provided so that, upon secretion and processing at the processing site, the desired protein will have the natural sequence. Specific examples of coding
20 sequences of interest for use in accordance with the present invention include the 12q23-qter polypeptide-coding sequences disclosed herein.

As previously mentioned, a marker may be present for selection of cells containing the vector construct. The marker may be an inducible or non-inducible gene and will generally allow for positive selection under
25 induction, or without induction, respectively. Examples of marker genes include neomycin, dihydrofolate reductase, glutamine synthetase, and the like. The vector employed will generally also include an origin of replication and other genes that are necessary for replication in the host cells, as routinely employed by those having skill in the art. As an example, the
30 replication system comprising the origin of replication and any proteins associated with replication encoded by a particular virus may be included as

part of the construct. The replication system must be selected so that the genes encoding products necessary for replication do not ultimately transform the cells. Such replication systems are represented by replication-defective adenovirus (see G. Acsadi et al., 1994, *Hum. Mol. Genet.* 3:579-584) and by Epstein-Barr virus. Examples of replication defective vectors, particularly, retroviral vectors that are replication defective, are BAG, (see Price et al., 1987, *Proc. Natl. Acad. Sci. USA*, 84:156; Sanes et al., 1986, *EMBO J.*, 5:3133). It will be understood that the final gene construct may contain one or more genes of interest, for example, a gene encoding a bioactive metabolic molecule. In addition, cDNA, synthetically produced DNA or chromosomal DNA may be employed utilizing methods and protocols known and practiced by those having skill in the art.

According to one approach for gene therapy, a vector encoding a 12q23-qter polypeptide is directly injected into the recipient cells (*in vivo* gene therapy). Alternatively, cells from the intended recipients are explanted, genetically modified to encode a 12q23-qter polypeptide, and reimplanted into the donor (*ex vivo* gene therapy). An *ex vivo* approach provides the advantage of efficient viral gene transfer, which is superior to *in vivo* gene transfer approaches. In accordance with *ex vivo* gene therapy, the host cells are first transfected with engineered vectors containing at least one gene encoding a 12q23-qter polypeptide, suspended in a physiologically acceptable carrier or excipient such as saline or phosphate buffered saline, and the like, and then administered to the host. The desired gene product is expressed by the injected cells, which thus introduce the gene product into the host. The introduced gene products can thereby be utilized to treat or ameliorate a disorder (e.g., asthma, obesity, or inflammatory bowel disease) that is related to altered levels of the 12q23-qter polypeptide.

ANIMAL MODELS

In accordance with the present invention, 12q23-qter polynucleotides (e.g., SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4687) can be used to generate genetically altered non-human animals or human cell lines. Any non-human animal can be used; however typical animals are rodents, such as mice, rats, or guinea pigs. Genetically engineered animals or cell lines can carry a gene that has been altered to contain deletions, substitutions, insertions, or modifications of the polynucleotide sequence (e.g., exon sequence). Such alterations may render the gene nonfunctional, (i.e., a null mutation) producing a "knockout" animal or cell line. In addition, genetically engineered animals can carry one or more exogenous or non-naturally occurring genes, i.e., "transgenes", that are derived from different organisms (e.g., humans), or produced by synthetic or recombinant methods. Genetically altered animals or cell lines can be used to study 12q23-qter gene function, regulation, and treatments for 12q23-qter-related diseases. In particular, knockout animals and cell lines can be used to establish animal models and *in vitro* models for 12q23-qter-related illnesses, respectively. In addition, transgenic animals expressing human 12q23-qter can be used in drug discovery efforts.

A "transgenic animal" is any animal containing one or more cells bearing genetic information altered or received, directly or indirectly, by deliberate genetic manipulation at a subcellular level, such as by targeted recombination or microinjection or infection with recombinant virus. The term "transgenic animal" is not intended to encompass classical cross-breeding or *in vitro* fertilization, but rather is meant to encompass animals in which one or more cells are altered by, or receive, a recombinant DNA molecule. This recombinant DNA molecule may be specifically targeted to a defined genetic locus, may be randomly integrated within a chromosome, or it may be extrachromosomally replicating DNA.

Transgenic animals can be selected after treatment of germline cells or zygotes. For example, expression of an exogenous 12q23-qter gene or a

variant can be achieved by operably linking the gene to a promoter and optionally an enhancer, and then microinjecting the construct into a zygote (see, e.g., Hogan et al., *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Such treatments include insertion of the exogenous gene and disrupted homologous genes. Alternatively, the gene(s) of the animals may be disrupted by insertion or deletion mutation of other genetic alterations using conventional techniques (see, e.g., Capecchi, 1989, *Science*, **244**:1288; Valancuis et al., 1991, *Mol. Cell Biol.*, **11**:1402; Hasty et al., 1991, *Nature*, **350**:243; Shinkai et al., 1992, *Cell*, **68**:855; Mombaerts et al., 1992, *Cell*, **68**:869; Philpott et al., 1992, *Science*, **256**:1448; Snouwaert et al., 1992, *Science*, **257**:1083; Donehower et al., 1992, *Nature*, **356**:215).

In one aspect of the invention, 12q23-qter gene knockout mice can be produced in accordance with well-known methods (see, e.g., M.R. Capecchi, 1989, *Science*, **244**:1288-1292; P. Li et al., 1995, *Cell* **80**:401-411; L.A. Galli-Taliadoros et al., 1995, *J. Immunol. Methods* **181**(1):1-15; C.H. Westphal et al., 1997, *Curr. Biol.* **7**(7):530-3; S.S. Cheah et al., 2000, *Methods Mol. Biol.* **136**:455-63). The disclosed murine 12q23-qter genomic clone can be used to prepare a 12q23-qter targeting construct that can disrupt 12q23-qter in the mouse by homologous recombination at the 12q23-qter chromosomal locus. The targeting construct can comprise a disrupted or deleted 12q23-qter gene sequence that inserts in place of the functioning portion of the native mouse gene. For example, the construct can contain an insertion in the 12q23-qter protein-coding region.

Preferably, the targeting construct contains markers for both positive and negative selection. The positive selection marker allows the selective elimination of cells that lack the marker, while the negative selection marker allows the elimination of cells that carry the marker. In particular, the positive selectable marker can be an antibiotic resistance gene, such as the neomycin resistance gene, which can be placed within the coding sequence of a 12q23-qter gene to render it non-functional, while at the same time

rendering the construct selectable. The herpes simplex virus thymidine kinase (HSV tk) gene is an example of a negative selectable marker that can be used as a second marker to eliminate cells that carry it. Cells with the HSV tk gene are selectively killed in the presence of gangcyclovir. As an example, a positive selection marker can be positioned on a targeting construct within the region of the construct that integrates at the locus of the 12q23-qter gene. The negative selection marker can be positioned on the targeting construct outside the region that integrates at the locus of the 12q23-qter gene. Thus, if the entire construct is present in the cell, both positive and negative selection markers will be present. If the construct has integrated into the genome, the positive selection marker will be present, but the negative selection marker will be lost.

The targeting construct can be employed, for example, in embryonal stem cell (ES). ES cells may be obtained from pre-implantation embryos cultured *in vitro* (M.J. Evans et al., 1981, *Nature* 292:154-156; M.O. Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:9065-9069; Robertson et al., 1986, *Nature* 322:445-448; S. A. Wood et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4582-4584). Targeting constructs can be efficiently introduced into the ES cells by standard techniques such as DNA transfection or by retrovirus-mediated transduction. Following this, the transformed ES cells can be combined with blastocysts from a non-human animal. The introduced ES cells colonize the embryo and contribute to the germ line of the resulting chimeric animal (R. Jaenisch, 1988, *Science* 240:1468-1474). The use of gene-targeted ES cells in the generation of gene-targeted transgenic mice has been previously described (Thomas et al., 1987, *Cell* 51:503-512) and is reviewed elsewhere (Frohman et al., 1989, *Cell* 56:145-147; Capecchi, 1989, *Trends in Genet.* 5:70-76; Baribault et al., 1989, *Mol. Biol. Med.* 6:481-492; Wagner, 1990, *EMBO J.* 9:3025-3032; Bradley et al., 1992, *Bio/Technology* 10: 534-539).

Several methods can be used to select homologously recombined murine ES cells. One method employs PCR to screen pools of transformant

cells for homologous insertion, followed by screening individual clones (Kim et al., 1988, *Nucleic Acids Res.* 16:8887-8903; Kim et al., 1991, *Gene* 103:227-233). Another method employs a marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly (Sedivy et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:227-231). For example, the positive-negative selection (PNS) method can be used as described above (see, e.g., Mansour et al., 1988, *Nature* 336:348-352; Capecchi, 1989, *Science* 244:1288-1292; Capecchi, 1989, *Trends in Genet.* 5:70-76). In particular, the PNS method is useful for targeting genes that are expressed at low levels.

The absence of functional 12q23-qter gene in the knockout mice can be confirmed, for example, by RNA analysis, protein expression analysis, and functional studies. For RNA analysis, RNA samples are prepared from different organs of the knockout mice and the 12q23-qter transcript is detected in Northern blots using oligonucleotide probes specific for the transcript. For protein expression detection, antibodies that are specific for the 12q23-qter polypeptide are used, for example, in flow cytometric analysis, immunohistochemical staining, and activity assays. Alternatively, functional assays are performed using preparations of different cell types collected from the knockout mice.

Several approaches can be used to produce transgenic mice. In one approach, a targeting vector is integrated into ES cell by homologous recombination, an intrachromosomal recombination event is used to eliminate the selectable markers, and only the transgene is left behind (A.L. Joyner et al., 1989, *Nature* 338(6211):153-6; P. Hasty et al., 1991, *Nature* 350(6315):243-6; V. Valancius and O. Smithies, 1991, *Mol. Cell Biol.* 11(3):1402-8; S. Fiering et al., 1993, *Proc. Natl. Acad. Sci. USA* 90(18):8469-73). In an alternative approach, two or more strains are created; one strain contains the gene knocked-out by homologous recombination, while one or more strains contain transgenes. The knockout strain is crossed with the transgenic strain to produce new line of animals in

which the original wild-type allele has been replaced (although not at the same site) with a transgene. Notably, knockout and transgenic animals can be produced by commercial facilities (e.g., The Lerner Research Institute, Cleveland, OH; B&K Universal, Inc., Fremont, CA; DNX Transgenic Sciences, Cranbury, NJ; Incyte Genomics, Inc., St. Louis, MO).

Transgenic animals (e.g., mice) containing a nucleic acid molecule which encodes a human 12q23-qter polypeptide, may be used as *in vivo* models to study the overexpression of a 12q23-qter gene. Such animals can also be used in drug evaluation and discovery efforts to find compounds effective to inhibit or modulate the activity of a 12q23-qter gene, such as for example compounds for treating respiratory disorders, diseases, or conditions. One having ordinary skill in the art can use standard techniques to produce transgenic animals which produce a human 12q23-qter polypeptide, and use the animals in drug evaluation and discovery projects (see, e.g., U.S. Patent No. 4,873,191 to Wagner; U.S. Patent No. 4,736,866 to Leder).

In another embodiment of the present invention, the transgenic animal can comprise a recombinant expression vector in which the nucleotide sequence that encodes a human 12q23-qter polypeptide is operably linked to a tissue specific promoter whereby the coding sequence is only expressed in that specific tissue. For example, the tissue specific promoter can be a mammary cell specific promoter and the recombinant protein so expressed is recovered from the animal's milk.

In yet another embodiment of the present invention, a 12q23-qter gene "knockout" can be produced by administering to the animal antibodies (e.g., neutralizing antibodies) that specifically recognize an endogenous 12q23-qter polypeptide. The antibodies can act to disrupt function of the endogenous 12q23-qter polypeptide, and thereby produce a null phenotype. In one specific example, an orthologous mouse 12q23-qter polypeptide or peptide can be used to generate antibodies. These antibodies can be given to a mouse to knockout the function of the mouse 12q23-qter ortholog.

In another embodiment of the present invention, non-mammalian organisms may be used to study 12q23-qter genes and 12q23-qter-related diseases. In particular, model organisms such as *C. elegans*, *D. melanogaster*, and *S. cerevisiae* may be used. Orthologs of 12q23-qter genes can be identified in these model organisms, and mutated or deleted to produce strains deficient for 12q23-qter genes. Human 12q23-qter genes can then be tested for the ability to "complement" the deficient strains. Such strains can also be used for drug screening. The 12q23-qter orthologs can be used to facilitate the understanding of the biological function of the human 12q23-qter genes, and assist in the identification of binding factors (e.g., agonists, antagonists, and inhibitors).

GENE IDENTIFICATION

To identify genes in the region on 12q23-qter, a set of bacterial artificial chromosome (BAC) clones containing this chromosomal region was identified. The BAC clones served as a template for genomic DNA sequencing and as reagents for identifying coding sequences by direct cDNA selection. Genomic sequencing and direct cDNA selection were used to characterize DNA from 12q23-qter in accordance with the methods described in detail herein.

When a gene has been genetically localized to a specific chromosomal region, the genes in this region can be characterized at the molecular level by a series of steps that include: (1) cloning the entire region of DNA in a set of overlapping genomic clones (physical mapping); (2) characterizing the genes encoded by these clones by a combination of direct cDNA selection, exon trapping and DNA sequencing (gene identification); and (3) identifying mutations in these genes by comparative DNA sequencing of affected and unaffected members of the kindreds and/or in unrelated affected individuals and unrelated unaffected controls (mutation analysis).

Physical mapping is accomplished by screening libraries of human DNA cloned in vectors that are propagated in a host such as *E. coli*, using

hybridization or PCR assays from unique molecular landmarks in the chromosomal region of interest. To generate a physical map of the disorder region, a library of human DNA cloned in BACs was screened with a set of overgo markers that had been previously mapped to chromosome 12q23-qter by the efforts of the Human Genome Project. Overgos are unique molecular landmarks in the human genome that can be assayed by hybridization. Through the combined efforts of the Human Genome Project, the location of thousands of overgos on the twenty-two autosomes and two sex chromosomes has been determined. For a positional cloning effort, the physical map is tied to the genetic map because the markers used for genetic mapping can also be used as overgos for physical mapping. By screening a BAC library with a combination of overgos derived from genetic markers, genes, and random DNA fragments, a physical map comprised of overlapping clones representing all of the DNA in a chromosomal region of interest can be assembled.

BACs are cloning vectors for large (80 kilobase to 200 kilobase) segments of human or other DNA that are propagated in *E. coli*. To construct a physical map using BACs, a library of BAC clones is screened so that individual clones harboring the DNA sequence corresponding to a given overgo or set of overgos are identified. Throughout most of the human genome, the overgo markers are spaced approximately 20 to 50 kilobases apart, so that an individual BAC clone typically contains at least two overgo markers. In addition, the BAC libraries that were screened contain enough cloned DNA to cover the human genome twelve times over. Accordingly, an individual overgo typically identifies more than one BAC clone. By screening a twelve-fold coverage BAC library with a series of overgo markers spaced approximately 50 kilobases apart, a physical map consisting of a series of overlapping contiguous BAC clones, i.e., BAC "contigs," can be assembled for any region of the human genome. This map is closely tied to the genetic map because many of the overgo markers used to prepare the physical map are also genetic markers.

When constructing a physical map, it often happens that there are gaps in the overgo map of the genome that result in the inability to identify BAC clones that are overlapping in a given location. Typically, the physical map is first constructed from a set of overgos identified through the publicly available literature and World Wide Web resources. The initial map consists of several separate BAC contigs that are separated by gaps of unknown molecular distance. To identify BAC clones that fill these gaps, it is necessary to develop new overgo markers from the ends of the clones on either side of the gap. This is done by sequencing the terminal 200 to 300 base pairs of the BACs flanking the gap, and developing a PCR or hybridization based assay. If the terminal sequences are demonstrated to be unique within the human genome, then the new overgo can be used to screen the BAC library to identify additional BACs that contain the DNA from the gap in the physical map. To assemble a BAC contig that covers a region the size of the disorder region (6,000,000 or more base pairs), it is necessary to develop new overgo markers from the ends of a number of clones.

After building a BAC contig, this set of overlapping clones serves as a template for identifying the genes encoded in the chromosomal region. Gene identification can be accomplished by many methods. Three methods are commonly used: (1) a set of BACs selected from the BAC contig to represent the entire chromosomal region can be sequenced, and computational methods can be used to identify all of the genes, (2) the BACs from the BAC contig can be used as a reagent to clone cDNAs corresponding to the genes encoded in the region by a method termed direct cDNA selection, or (3) the BACs from the BAC contig can be used to identify coding sequences by selecting for specific DNA sequence motifs in a procedure called exon trapping. The present invention includes chromosome 12q23-qter genes identified by the first two methods.

To sequence the entire BAC contig representing the disorder region, a set of BACs can be chosen for subcloning into plasmid vectors and

subsequent DNA sequencing of these subclones. Since the DNA cloned in the BACs represents genomic DNA, this sequencing is referred to as genomic sequencing to distinguish it from cDNA sequencing. To initiate the genomic sequencing for a chromosomal region of interest, several non-overlapping BAC clones are chosen. DNA for each BAC clone is prepared, and the clones are sheared into random small fragments, which are subsequently cloned into standard plasmid vectors such as pUC18. The plasmid clones are then grown to propagate the smaller fragments, and these are the templates for sequencing. To ensure adequate coverage and sequence quality for the BAC DNA sequence, sufficient plasmid clones are sequenced to yield three-fold coverage of the BAC clone. For example, if the BAC is 100 kilobases long, then phagemids are sequenced to yield 300 kilobases of sequence. Since the BAC DNA was randomly sheared prior to cloning in the phagemid vector, the 300 kilobases of raw DNA sequence can be assembled by computational methods into overlapping DNA sequences termed sequence contigs. For the purposes of initial gene identification by computational methods, three-fold coverage of each BAC is sufficient to yield twenty to forty sequence contigs of 1000 base pairs to 20,000 base pairs.

The sequencing strategy employed in this invention was to initially sequence "seed" BACs from the BAC contig in the disorder region. The sequence of the "seed" BACs was then used to identify minimally overlapping BACs from the contig, and these were subsequently sequenced. In this manner, the entire candidate region can be sequenced, with several small sequence gaps left in each BAC. This sequence serves as the template for computational gene identification.

In one approach, genes can be identified by comparing the sequence of BAC contig to publicly available databases of cDNA and genomic sequences, e.g., UniGene, dbEST, EMBL nucleotide database, GenBank, and the DNA Database of Japan (DDBJ). The BAC DNA sequence can also be translated into protein sequence, and the protein sequence can be used

to search publicly available protein databases, e.g., GenPept, EMBL protein database, Protein Information Resource (PIR), Protein Data Bank (PDB), and SWISS-PROT. These comparisons are typically done using the BLAST family of computer algorithms and programs (Altschul et al., 1990, *J. Mol. Biol.*, 215:403-410; Altschul et al, 1997, *Nucl. Acids Res.*, 25:3389-3402).

For nucleotide queries, BLASTN, BLASTX, and TBLASTX can be used. BLASTN compares a nucleotide query sequence with a nucleotide sequence database; BLASTX compares a nucleotide query sequence translated in all reading frames against a protein sequence database; TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. For protein queries, BLASTP and TBLASTN can be used. BLASTP compares a protein query sequence with a protein sequence database; TBLASTN compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames.

Additionally, computer algorithms such as MZEF (Zhang, 1997, *Proc. Natl. Acad. Sci. USA* 94:565-568), GRAIL (Uberbacher et al., 1996, *Methods Enzymol.* 266:259-281), and Genscan (Burge and Karlin, 1997, *J. Mol. Biol.*, 268:78-94) can be used to predict the location of exons in the sequence based on the presence of specific DNA sequence motifs that are common to all exons, as well as the presence of codon usage typical of human protein encoding sequences.

In addition to identifying genes by computational methods, genes can be identified by direct cDNA selection (Del Mastro and Lovett, 1996, *Methods in Molecular Biology*, Humana Press Inc., NJ). In direct cDNA selection, cDNA pools from tissues of interest are prepared, and BACs from the candidate region are used in a liquid hybridization assay to capture the cDNAs which base pair to coding regions in the BAC. In the methods described herein, the cDNA pools were created from several different tissues by random priming and oligo dT priming the first strand cDNA from poly A⁺ RNA, synthesizing the second-strand cDNA by standard methods,

- and adding linkers to the ends of the cDNA fragments. In this approach, the linkers are used to amplify the cDNA pools of BAC clones from the disorder region identified by screening a BAC library. The amplified products are then used as a template for initiating DNA synthesis to create a biotin
- 5 labeled copy of BAC DNA. Following this, the biotin labeled copy of the BAC DNA is denatured and incubated with an excess of the PCR amplified, linkered cDNA pools which have also been denatured. The BAC DNA and cDNA are allowed to anneal in solution, and heteroduplexes between the BAC and the cDNA are isolated using streptavidin coated magnetic beads.
- 10 The cDNAs that are captured by the BAC are then amplified using primers complimentary to the linker sequences, and the hybridization/selection process is repeated for a second round. After two rounds of direct cDNA selection, the cDNA fragments are cloned, and a library of these direct selected fragments is created.
- 15 The cDNA clones isolated by direct selection are analyzed by two methods. Since a pool of BACs from the disorder region is used to provide the genomic target DNA sequence, the cDNAs must be mapped to BAC genomic clones to verify their chromosomal location. This is accomplished by arraying the cDNAs in microtiter dishes, and replicating their DNA in high-
- 20 density grids. Individual genomic clones known to map to the region are then hybridized to the grid to identify direct selected cDNAs mapping to that region. cDNA clones that are confirmed to correspond to individual BACs are sequenced. To determine whether the cDNA clones isolated by direct selection share sequence identity or similarity to previously identified genes,
- 25 the DNA and protein coding sequences are compared to publicly available databases using the BLAST family of programs.

The combination of genomic DNA sequence and cDNA sequence provided by BAC sequencing and by direct cDNA selection yields an initial list of putative genes in the region. The genes in the region were all

30 candidates for the asthma locus. To further characterize each gene, Northern blots were performed to determine the size of the transcript

- corresponding to each gene, and to determine which putative exons were transcribed together to make an individual gene. For Northern blot analysis of each gene, probes were prepared from direct selected cDNA clones or by PCR amplifying specific fragments from genomic DNA, cDNA or from the
- 5 BAC encoding the putative gene of interest. The Northern blots gave information on the size of the transcript and the tissues in which it was expressed. For transcripts that were not highly expressed, it was sometimes necessary to perform a reverse transcription PCR assay using RNA from the tissues of interest as a template for the reaction.
- 10 Gene identification by computational methods and by direct cDNA selection provides unique information about the genes in a region of a chromosome. When genes are identified, then it is possible to examine different individuals for mutations in each gene. Variants in gene sequences between individuals can be inherited allelic differences or can arise from
- 15 mutations in the individuals. Gene sequence variants are clinically important in that they can affect drug action on such gene. Most drugs elicit a safe response in only a fraction of individuals, and drugs are commonly administered to patients with no certainty that they will be safe and effective. Many important drugs are effective in only 30-40% of patients for whom the
- 20 drug is prescribed, and virtually all drugs cause adverse events in some individuals. Identification of mutations in disorder genes in different individuals will enable a correlation between the safety and efficacy of drug therapies used to treat lung diseases and the genotypes of the treated individuals. This correlation enables health care providers to prescribe a
- 25 drug regimen that is most appropriate for the individual patient rather than trying different drug regimens in turn until a successful drug is identified. Identification of variants in disorder genes will also have a benefit during the development of new drugs for the treatment of lung diseases, as the ability to correlate genetic variation with the efficacy of new candidate drugs will
- 30 enhance lead optimization and increase the efficiency and success rate of new drug approvals.

Gene identification by computational methods and by direct cDNA selection provides unique information about the genes in a region of a chromosome. Once genes are identified, it is possible to examine subjects for sequence variants. Variant sequences can be inherited as allelic differences or can arise from spontaneous mutations. Inherited alleles can be analyzed for linkage to a disease susceptibility locus. Linkage analysis is possible because of the nature of inheritance of chromosomes from parents to offspring. During meiosis, the two parental homologs pair to guide their proper separation to daughter cells. While they are paired, the two homologs exchange pieces of the chromosomes, in an event called "crossing over" or "recombination." The resulting chromosomes contain parts that originate from both parental homologs. The closer together two sequences are on the chromosome, the less likely that a recombination event will occur between them, and the more closely linked they are.

Data obtained from the different families can be combined and analyzed together by a computer using statistical methods described herein. The results can then be used as evidence for linkage between the genetic markers used and an asthma susceptibility locus. In general, a recombination frequency of 1% is equivalent to approximately 1 map unit, a relationship that holds up to frequencies of about 20% or 20 cM. One centimorgan (cM) is roughly equivalent to 1,000 Kb of DNA. The entire human genome is 3,300 cM long. In order to find an unknown disease gene within 5-10 cM of a marker locus, the whole human genome can be searched with roughly 330 informative marker loci spaced at approximately 10 cM intervals (Botstein et al., 1980, *Am. J. Hum. Genet.* 32:314-331).

The reliability of linkage results is established by using a number of statistical methods. The methods most commonly used for the detection by linkage analysis of oligogenes involved in the etiology of a complex trait are non-parametric or model-free methods which have been implemented into the computer programs MAPMAKER/SIBS (L. Kruglyak and E.S. Lander, 1995, *Am. J. Hum. Genet.* 57:439-454) and GENEHUNTER (L. Kruglyak et

al., 1996, *Am. J. Hum. Genet.* 58:1347-1363). Typically, linkage analysis is performed by typing members of families with multiple affected individuals at a given marker locus and evaluating if the affected members (excluding parent-offspring pairs) share alleles at the marker locus that are identical by descent (IBD) more often than expected by chance alone.

As a result of the rapid advances in mapping the human genome over the last few years, and concomitant improvements in computer methodology, it has become feasible to carry out linkage analyses using multi-point data. Multi-point analysis provides a simultaneous analysis of linkage between the trait and several linked genetic markers, when the recombination distance among the markers is known. A LOD score statistic is computed at multiple locations along a chromosome to measure the evidence that a susceptibility locus is located nearby. A LOD score is the logarithm base 10 of the ratio of the likelihood that a susceptibility locus exists at a given location to the likelihood that no susceptibility locus is located there. By convention, when testing a single marker, a total LOD score greater than +3.0 (that is, odds of linkage being 1,000 times greater than odds of no linkage) is considered to be significant evidence for linkage.

Multi-point analysis is advantageous for two reasons. First, the informativeness of the pedigrees is usually increased. Each pedigree has a certain amount of potential information, dependent on the number of parents heterozygous for the marker loci and the number of affected individuals in the family. However, few markers are sufficiently polymorphic as to be informative in all those individuals. If multiple markers are considered simultaneously, then the probability of an individual being heterozygous for at least one of the markers is greatly increased. Second, an indication of the position of the disease gene among the markers may be determined. This allows identification of flanking markers, and thus eventually allows identification of a small region in which the disease gene resides.

EXAMPLES

The examples as set forth herein are meant to exemplify the various aspects of the present invention and are not intended to limit the invention in any way.

5 EXAMPLE 1: FAMILY COLLECTION

Asthma is a complex disorder that is influenced by a variety of factors, including both genetic and environmental effects. Complex disorders are typically caused by multiple interacting genes, some contributing to disease development and some conferring a protective effect.

10 The success of linkage analyses in identifying chromosomes with significant LOD scores is achieved in part as a result of an experimental design tailored to the detection of susceptibility genes in complex diseases, even in the presence of epistasis and genetic heterogeneity. Also important are rigorous efforts in ascertaining asthmatic families that meet strict guidelines,

15 and collecting accurate clinical information.

Given the complex nature of the asthma phenotype, non-parametric affected sib pair analyses were used to analyze the genetic data. This approach does not require parameter specifications such as mode of inheritance, disease allele frequency, penetrance of the disorder, or

20 phenocopy rates. Instead, it determines whether the inheritance pattern of a chromosomal region is consistent with random segregation. If it is not, affected siblings inherit identical copies of alleles more often than expected by chance. Because no models for inheritance are assumed, allele-sharing methods tend to be more robust than parametric methods when analyzing

25 complex disorders. They do, however, require larger sample sizes to reach statistically significant results.

At the outset of the program, the goal was to collect 400 affected sib-pair families for the linkage analyses. Based on a genome scan with markers spaced ~10 cM apart, this number of families was predicted to

30 provide > 95% power to detect an asthma susceptibility gene that caused an increased risk to first-degree relatives of 3-fold or greater. The assumed

relative risk of 3-fold was consistent with epidemiological studies in the literature that suggest an increased risk ranging from 3- to 7-fold. The relative risk was based on gender, different classifications of the asthma phenotype (i.e., bronchial hyper-responsiveness versus physician's diagnosis) and, in the case of offspring, whether one or both parents were asthmatic.

The family collection efforts exceeded the initial goal of 400, and resulted in a total of 444 affected sibling pair (ASP) families, with 342 families from the UK and 102 families from the US. The ASP families in the US collection were Caucasian with a minimum of two affected siblings that were identified through both private practice and community physicians as well as through advertising. A total of 102 families were collected in Kansas, Nebraska, and Southern California. In the UK collection, Caucasian families with a minimum of two affected siblings were identified through physicians' registers in a region surrounding Southampton and including the Isle of Wight. In both the US and UK collections, additional affected and unaffected sibs were collected whenever possible.

An additional 63 families from the United Kingdom were utilized from an earlier collection effort with different ascertainment criteria. These families were recruited either: 1) without reference to asthma and atopy; or 2) by having at least one family member or at least two family members affected with asthma. The randomly ascertained samples were identified from general practitioner registers in the Southampton area. For families with affected members, the probands were recruited from hospital based clinics in Southampton. Seven pedigrees extended beyond a single nuclear family. The phenotypic and genotypic data information for 17 markers for 21 of these 63 families was obtained from the website <http://cedar.genetics.soton.ac.uk/pub/PROGRAMS/BETA/data/bet12.ped>.

Families were included in the study if they met all of the following criteria: 1) the biological mother and biological father were Caucasian and agreed to participate in the study; 2) at least two biological siblings were

alive, each with a current physician diagnosis of asthma, and were 5 to 21 years of age; and 3) the two siblings were currently taking asthma medications on a regular basis. This included regular, intermittent use of inhaled or oral bronchodilators and regular use of cromolyn, theophylline, or steroids.

Families were excluded from the study if they met any one of the following criteria: 1) both parents were affected (i.e., with a current diagnosis of asthma, having asthma symptoms, or on asthma medications at the time of the study); 2) any of the siblings to be included in the study was less than 5 years of age; 3) any asthmatic family member to be included in the study was taking beta-blockers at the time of the study, 4) any family member to be included in the study had congenital or acquired pulmonary disease at birth (e.g., cystic fibrosis), a history of serious cardiac disease (myocardial infarction), or any history of serious pulmonary disease (e.g., emphysema); or 5) any family member to be included in the study was pregnant.

An extensive clinical instrument was designed and data from all participating family members were collected. The case report form (CRF) included questions on demographics, medical history including medications, a health survey on the incidence and frequency of asthma, wheeze, eczema, hay fever, nasal problems, smoking, and questions on home environment. Data from a video questionnaire designed to show various examples of wheeze and asthmatic attacks were also included in the CRF. Clinical data, including skin prick tests to 8 common allergens, total and specific IgE levels, and bronchial hyper-responsiveness following a methacholine challenge, were also collected from all participating family members. All data were entered into a SAS dataset by IMTCI, a CRO; either by double data entry or scanning followed by on-screen visual validation. An extensive automated review of the data was performed on a routine basis and a full audit at the conclusion of the data entry was completed to verify the accuracy of the dataset.

EXAMPLE 2: GENOME SCAN

In order to identify chromosomal regions linked to asthma, the inheritance pattern of alleles from genetic markers spanning the genome was assessed on the collected family resources. As described above, combining these results with the segregation of the asthma phenotype in these families allows the identification of genetic markers that are tightly linked to asthma. In turn, this provides an indication of the location of genes predisposing affected individuals to asthma. The genotyping strategy was twofold: 1) to conduct a genome wide scan using markers spaced at approximately 10 cM intervals; and 2) to target ten chromosomal regions for high density genetic mapping. The initial candidate regions for high-density mapping were chosen based on suggestions of linkage to these regions by other investigators.

Genotypes of PCR amplified simple sequence microsatellite genetic linkage markers were determined using ABI model 377 Automated Sequencers (PE Applied Biosystems). Microsatellite markers were obtained from Research Genetics Inc. (Huntsville, AL) in the fluorescent dye-conjugated form (see Dubovsky et al., 1995, *Hum. Mol. Genet.* 4(3):449-452). The markers comprised a variation of a human linkage mapping panel as released from the Cooperative Human Linkage Center (CHLC), also known as the Weber lab screening set version 8. The variation of the Weber 8 screening set consisted of 529 markers with an average spacing of 6.9 cM (autosomes only) and 7.0 cM (all chromosomes). Eighty-nine percent of the markers consisted of either tri- or tetra-nucleotide microsatellites. There were no gaps present in chromosomal coverage greater than 17.5 cM.

Study subject genomic DNA (5 µl; 4.5 ng/µl) was amplified in a 10 µl PCR reaction using AmpliTaq Gold DNA polymerase (0.225 U); 1 X PCR buffer (80 mM (NH₄)₂SO₄; 30 mM Tris-HCl (pH 8.8); 0.5% Tween-20); 200 µM each dATP, dCTP, dGTP and dTTP; 1.5-3.5 µM MgCl₂; and 250 µM forward and reverse PCR primers. PCR reactions were set up in 192 well

plates (Costar) using a Tecan Genesis 150 robotic workstation equipped with a refrigerated deck. PCR reactions were overlaid with 20 μ l mineral oil, and thermocycled on an MJ Research Tetrad DNA Engine equipped with four 192 well heads using the following conditions: 92°C for 3 min; 6 cycles of 92°C for 30 sec, 56°C for 1 min, 72°C for 45 sec; followed by 20 cycles of 92°C for 30 sec, 55°C for 1 min, 72°C for 45 sec; and a 6 min incubation at 72°C.

PCR products of 8-12 microsatellite markers were subsequently pooled into two 96-well microtitre plates (2.0 μ l PCR product from TET and FAM labeled markers, 3.0 μ l HEX labeled markers) using a Tecan Genesis 200 robotic workstation and brought to a final volume of 25 μ l with H₂O. Following this, 1.9 μ l of pooled PCR product was transferred to a loading plate and combined with 3.0 μ l loading buffer (2.5 μ l formamide/blue dextran (9.0 mg/ml), 0.5 μ l GS-500 TAMRA labeled size standard, ABI). Samples were denatured in the loading plate for 4 min at 95°C, placed on ice for 2 min, and electrophoresed on a 5% denaturing polyacrylamide gel (FMC on the ABI 377XL). Samples (0.8 μ l) were loaded onto the gel using an 8 channel Hamilton Syringe pipettor.

Each gel consisted of 62 study subjects and 2 control subjects (CEPH parents ID #1331-01 and 1331-02, Coriell Cell Repository, Camden, NJ). Genotyping gels were scored in duplicate by investigators blind to patient identity and affection status using GENOTYPER analysis software V 1.1.12 (ABI; PE Applied Biosystems). Nuclear families were loaded onto the gel with the parents flanking the siblings to facilitate error detection. The final tables obtained from the GENOTYPER output for each gel analysed were imported into a SYBASE Database.

Allele calling (binning) was performed using the SYBASE version of the ABAS software (Ghosh et al., 1997, *Genome Research* 7:165-178). Offsize bins were checked manually and incorrect calls were corrected or blanked. The binned alleles were then imported into the program MENDEL (Lange et al., 1988, *Genetic Epidemiology*, 5:471) for inheritance checking

using the USERM13 subroutine (Boehnke et al., 1991, *Am. J. Hum. Genet.* 48:22-25). Non-inheritance was investigated by examining the genotyping traces and, once all discrepancies were resolved, the subroutine USERM13 was used to estimate allele frequencies.

5 **EXAMPLE 3: LINKAGE ANALYSIS**

Chromosomal regions harboring asthma susceptibility genes were identified by linkage analysis of genotyping data and three separate phenotypes, asthma, bronchial hyper-responsiveness, and atopic status.

1. **Asthma Phenotype:** For the initial linkage analysis, the phenotype and asthma affection status were defined by a patient who answered the following questions in the affirmative: i) Have you ever had asthma? ii) Do you have a current physician's diagnosis of asthma? and iii) Are you currently taking asthma medications? Medications included inhaled or oral bronchodilators, cromolyn, theophylline, or steroids. Multipoint linkage analyses of allele sharing in affected individuals were performed using the MAPMAKER/SIBS analysis program (L. Kruglyak and E.S. Lander, 1995, *Am. J. Hum. Genet.* 57:439-454). The analyses were performed using 54 polymorphic markers spanning a 162 cM region on both arms of chromosome 12. The map location and distances between markers were obtained from the genetic maps published by the Marshfield medical research foundation (<http://www.marshmed.org/genetics/>). Ambiguous ordering of markers in the Marshfield map was resolved using the program MULTIMAP (T.C. Matise et al., 1994, *Nature Genet.* 6:384-390).

Figure 1A shows the multipoint LOD score against the map location of markers along chromosome 12. A Maximum LOD Score (MLS) of 2.9, based on 484 nuclear families, was obtained at location 161.7 cM, 1.0 cM distal to markers D12S97 and D12S1045. An excess sharing by descent (Identity By Descent; IBD=2) of 0.31 was observed at the MLS. Table 1B shows the two-point and multipoint LOD scores at each marker.

TABLE 1B: CHROMOSOME 12 LINKAGE ANALYSIS

Marker	Distance	Two-point	Multipoint
D12S372	6.4	0.0	0.0
GATA49D12	17.7	0.0	0.0
D12S77	20.3	0.0	0.0
D12S391	26.2	0.0	0.0
D12S358	26.2	0.0	0.0
D12S364	30.6	0.2	0.0
D12S373	36.1	0.0	0.0
D12S1042	48.7	0.0	0.0
GATA91H06	56.3	0.0	0.0
D12S368	66.0	0.2	0.3
D12S398	68.2	0.2	0.4
D12S83	75.2	1.1	0.0
D12S1294	78.1	0.0	0.0
IFNgama	80.4	0.0	0.0
D12S375	80.5	0.3	0.0
D12S43	80.5	0.3	0.0
D12S1052	83.2	0.0	0.0
D12S92	83.2	1.0	0.0
D12S326	86.4	0.1	0.1
D12S64	89.4	0.0	0.2
D12S379	93.7	0.0	0.1
D12S311	94.5	0.1	0.0
D12S82	95.0	0.1	0.1
D12S819	95.0	0.0	0.1
D12S1064	95.0	0.0	0.0
D12S95	96.1	0.2	0.2
D12S829	97.2	0.1	0.6
D12S1706	104.1	0.6	0.4
D12S1300	104.1	0.2	0.3
D12S1727	107.2	0.0	0.1
D12S1607	107.9	0.0	0.1
IGF1	109.5	0.0	0.0
PAH	109.5	0.0	0.0
D12S360	111.3	0.0	0.0
D12S338	111.9	0.0	0.0
D12S78	111.9	0.0	0.0

D12S811	120.7	0.1	0.3
D12S1341	123.0	0.0	0.5
NOS1	123.1	0.1	0.4
D12S2070	125.3	0.2	0.7
D12S366	133.3	1.2	1.7
D12S1619	134.5	0.8	1.8
D12S385	135.1	2.0	1.6
PLA2G1B	136.8	0.9	1.4
D12S395	136.8	2.1	1.5
D12S300	140.2	0.9	1.7
D12S342	144.8	1.6	2.2
D12S324	147.2	1.3	1.4
D12S2078	149.6	0.9	1.9
D12S1659	155.9	0.3	1.6
D12S97	160.7	0.9	2.7
D12S1045	160.7	3.0	2.8
D12S392	165.7	1.1	2.3
D12S357	168.8	0.8	1.1

2. Phenotypic Subgroups: Nuclear families were ascertained by the presence of at least two affected siblings with a current physician's diagnosis of asthma, as well as the use of asthma medication. In the initial analysis (see above), the evidence was examined for linkage based on that dichotomous phenotype (asthma – yes/no). To further characterize the linkage signals, additional quantitative traits were measured in the clinical protocol. Since quantitative trait loci (QTL) analysis tools with correction for ascertainment were not available, the following approach was taken to refine the linkage and association analyses:
- i. Phenotypic subgroups that could be indicative of an underlying genotypic heterogeneity were identified. Asthma subgroups were defined according to 1) bronchial hyper-responsiveness (BHR) to methacholine challenge; or 2) atopic status using quantitative measures like total serum IgE and specific IgE to common allergens.
 - ii. Non-parametric linkage analyses were performed on subgroups to test for the presence of a more homogeneous sub-sample. If

genetic heterogeneity was present in the sample, the amount of allele sharing among phenotypically similar siblings was expected to increase in the appropriate subgroup in comparison to the full sample. A narrower region of significant increased allele sharing was also expected to result unless the overall LOD score decreased as a consequence of having a smaller sample size and of using an approximate partitioning of the data.

3. Results for BHR and IgE: PC₂₀, the concentration of methacholine resulting in a 20% drop in FEV₁ (forced expiratory volume), was polychotomized into four groups and analyses were performed on the subsets of asthmatic children with borderline to severe BHR (PC₂₀ ≤ 16 mg/ml) or PC₂₀(16). As shown in the LOD plot in Figure 1B, the MLS for the subset of 218 nuclear families with at least two PC₂₀(16) affected sibs was 2.2 at D12S342 with an excess sharing of 0.33. The linkage results implicated a region of chromosome 12 centromeric to the region with the largest signal under the asthma phenotype (Figure 1A), and indicated the presence of one or more genes with specific susceptibility toward BHR. Since the BHR sample represented a subset of the sample of asthmatics, it elucidated the presence of multiple peaks in the LOD plot of Figure 1A.

Total IgE was dichotomized using an age specific cutoff for elevated levels (one standard deviation above the mean: 52 kU/L for age 5-9; 63 kU/L for age 10-14; 75 kU/L for age 15-18; and 81 kU/L for adults). Similarly, a dichotomous variable was created using specific IgE to common allergens. An individual was assigned a high specific IgE value if his/her level was positive (grass or tree) or elevated (> 0.35 KU/L for cat, dog, mite A, mite B, alternaria, or ragweed) for at least one such measure.

In linkage analyses, the subset of asthmatic children with high total IgE (274 families) gave a maximum LOD score of 2.3 at D12S1619 (Figure 1C) with an excess sharing of 0.33. The subset with high specific IgE (288 families) gave a LOD score of 2.2 at 164.2 cM, 1.5cM proximal to marker D12S392 with an excess sharing of 0.33 (Figure 1D). The analysis with the subset of asthmatic sibs with elevated total IgE implicated a region similar to

the one identified with the BHR subset. The region implicated by the subset of asthmatic with elevated specific IgE coincided with the location of the largest signal in the original asthma sample.

Accordingly, a pattern of evidence by linkage analysis pointed to the existence of several asthma susceptibility loci in the 12q23-ter region of chromosome 12. This was supported by the initial analysis of the asthma (yes/no) phenotype with further localization by analyses of BHR, total IgE, and specific IgE in asthmatic individuals. Thus, chromosome 12q23-ter encompassed genes involved in asthma and related diseases thereof.

10 **EXAMPLE 4: PHYSICAL MAPPING**

The linkage results for chromosome 12 described above were used to delineate a candidate region for disorder-associated gene(s) located on chromosome 12. Gene discovery efforts were initiated in a ~43 cM interval from marker D12S2070 to the 12q telomere, representing a 99% confidence interval. All genes known to map to this interval were considered candidates. Figures 2A-2P show genes mapped against the GB4 panel and Figures 3A-3G show genes mapped against the Stanford G3 panel. The figures were obtained directly from the GeneMap99 web site.

Physical mapping (BAC contig construction) focused on a ~22 cM interval approximately between markers D12S307 and D12S2341. The discovery of novel genes using direct cDNA selection focused on a ~15 cM region between markers D12S1609 and D12S357. Figure 4 shows the integration of the Marshfield Center for Medical Genetics (<http://www.marshmed.org/genetics/>) genetic map with GeneMap99 from NCBI. The relevant regions are indicated at the top of the figure.

The following section describes the construction of a BAC contig spanning the disorder gene region on chromosome 12. This approach was used: 1) to provide genomic clones for DNA sequencing (analysis of this sequence would provide information about the gene content of the region); and 2) to provide reagents for direct cDNA selection (and provide additional information about novel genes mapping to the interval). The physical map

consisted of an ordered set of molecular landmarks, and a set of BACs (U.-J. Kim et al., 1996, *Genomics* 34:213-218; H. Shizuya et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:8794-8797) that contained the disorder gene region from human chromosome 12q23-qter.

5 Figures 5A-5I show the BAC/STS content contig map of human chromosome 12q23-qter. Markers used to screen the RPCI-11 BAC library (P. deJong, Roswell Park Cancer Institute (RPCI)) are shown in the top row. Markers that were present in the Genome Database (GDB, <http://gdbwww.gdb.org/>) are represented by GDB nomenclature. The BAC clones are shown below the markers as horizontal lines.

10 1. Map Integration. Various publicly available mapping resources were utilized to identify existing STS (sequence tagged site) markers in the 12q23-qter region (Olson et al., 1989, *Science*, 245:1434-1435). Resources included GDB (<http://gdbwww.gdb.org/>), Genethon (http://www.genethon.fr/genethon_en.html), the Marshfield Center for Medical Genetics (<http://www.marshmed.org/genetics/>), the Whitehead Institute Genome Center (<http://www-genome.wi.mit.edu/>), GeneMap98, dbSTS, and dbEST (NCBI, <http://www.ncbi.nlm.nih.gov/>), the Sanger Centre (<http://www.sanger.ac.uk/>), and the Stanford Human Genome Center (<http://www-shgc.stanford.edu/>). Maps were integrated manually to identify markers mapping to the disorder region. A list of markers is shown in Table 2.

20 2. Marker Development: Sequences for existing STSs were obtained from the GDB, RHDB (<http://www.ebi.ac.uk/RHdb/>), or NCBI, and were used to pick primer pairs (overgos; see Table 2) for BAC library screening. Novel markers were developed from publicly available genomic sequences, proprietary cDNA sequences, or from sequences derived from BAC insert ends (described below). Primers were chosen using a script that automatically performs vector and repetitive sequence masking using CROSSMATCH (P. Green, University of Washington). Subsequent primer selection was performed using a customized Filemaker Pro database (<http://www.filemaker.com>). Primers for use in PCR-based clone

TABLE 2: PRIMER PAIRS

Marker name	Locus	DNA type	Gene	Forward primer	Seq ID NO.	Reverse primer	Seq ID NO.
B0610N03-A1.x		BACend		CAAGCGATAGTTCTAATTTCT		TATGTGTTGGAGCCAGAAATTT	
B0600D18-A2.x		BACend		TGGTGTCTCTGAGCTTCAGG		ACCGAAACCAAGATCCTGGAAG	
B0611O14-A2.x		BACend		GTCITGATTTAAGTTTGAGG		CTGCCCTCACCTTGCTCAAAC	
B0700A09-A2.x		BACend		GCTGCTCCAGCATTTTCAGAT		CAGTGTATATGATGCTGAA	
B0716I10-A2.x		BACend		ATGATGCAAGTGAGTGAGACCCA		CTTACTCACTACACTGGGCTC	
B1118B13-A2.x		BACend		GCACCTGGGCTTCTCATCTGCT		ACTCTCGTGGATAGAGCAGATG	
B1128N10-A2.x		BACend		CACGAGAGTCTAGTGGGGTTT		TCACCTGGCAGATGAACCCCC	
B0841C17-A2.x		BACend		TCCCTGATATCCACTATCTTT		CATTAGATGATGGTAAAGATAG	
B0904G06-A2.x		BACend		ACTGTCTCATTTCTTTACAGAA		GGAACAGCAACGTTTCTGTA	
B0923J13-A2.x		BACend		CAGGTCTCTGCAGAGCATTTCT		GACTCTTTTAAAGAGAAATGC	
B0675M15-A2.x		BACend		GCAGACAATATCAAGAGTTCTT		CTGTAAACACATCTCAAGAACTC	
B0600D16-A2.y		BACend		TCATCTGCCAAGTGAGCCAGT		GACCTCACCAAGCAGCTGGGCT	
B0610N03-A2.y		BACend		GATACCAATGTGAAGTCTTGA		GTTTCTCCAGCCTCAAGGAC	
B0700A09-A2.y		BACend		TCTGATCCCACTAACCACGAT		ATGAAGTACATTGGATCGTGGT	
B1118B13-A2.y		BACend		ACTGGAATGCTCAGCTGGATGC		TTCTCCAGGGTCAAGCATCCAG	
B1128N10-A2.y		BACend		TGCTGATCTCTCAGTTCCACCT		GCAAGCCACCCATCAGGGTGAA	
B0904G06-A2.y		BACend		ATCTAATGCTGTGGCCGCTGCT		GGTTTGTGTTGCTGCAGCAGCGG	
B0923J13-A2.y		BACend		GACAGCCAGAGGAACCTCTTC		AAAGTTGTCTTGGGAAGAGGT	
B0675M15-A2.y		BACend		CACCTCTGGCTTTCTCTACAACC		AGCTGTGACATGAAGGTGTAG	
B0635H04-A1.x		BACend		AGCTTCGCTGACCAGCTACCC		TTTCCAGGAACCAAGGTAGACT	
B0688B20-A1.x		BACend		TGCCTGTGACTGAAGTCTGAT		GAGTGAGTAAGGAATCAAGAC	
B0688D03-A1.x		BACend		AGGAAGAACAAGACGAGCTTT		GTCAATTATTCCTCAAGACTG	
B0700H07-A1.x		BACend		TCCTGGGAAGCAAGATAGGAA		TCCGAGTGGCTTTGTTCTATT	
B0726A20-A1.x		BACend		ACTGTGTCACCTCTCGGAAAG		AGCTTCCAGGCTCTTTCCCA	
B0761L21-A1.x		BACend		GAGTAAAGAATGTGTATAGGG		TTTTTGTACCCACCCCTATAC	

B0814G08-A1.x	BACend	CGAGGAAGATGTAAGAGACTGT	ATTGAGGCCCCAGAACAGTCTC	
B0857A05-A1.x	BACend	TCITTAGTCCTTTGGGAGAGCT	ATTTTCCACAGGAAGCTCTCC	
B0895C23-A1.x	BACend	AGGTGCTACCTCGCTCAATCTG	GGGCTGGTTGCTCAGAGATTGA	
B0849E15-A1.x	BACend	CTTTTGAAGAGGTGGTTCGT	GAATGCAAGCACTCAGAAACC	
B0804M18-A1.x	BACend	AGCCATAACACACATTTCTAT	GATGCTCTGTGCATATAGAAAT	
B0815D12-A1.x	BACend	TCCACTGAGAGTTACCAACCCC	GGTATGAGAATTTGGGTTTGG	
B0833K01-A1.x	BACend	GTTGAGATTTTATCTTGGTAT	ACTGATGACATTTGATACCCAA	
B0863H23-A1.x	BACend	GAGTGCCCTATTGCTGTGTTTT	CAGCCAAATGAAGTCAAAACACA	
B0898L08-A1.x	BACend	ATCTGTAGCCTATAGTGAACAG	TTTACAGTGTTCCTGTTTAC	
B0702C13-A1.x	BACend	GTAGTAACAGAATGGACTTTGA	AGAGAGGAACAGCATCAAAAGTC	
B0702F18-A1.x	BACend	CTCTGCAATTTCTTACTCCTTAC	AAGCTTTACTACCAGTAAGGAG	
B0728K24-A1.x	BACend	TCGCAATAGCACAGGGACIT	CACCGTTATGCAGAAAGTCCCT	
B0738O20-A1.x	BACend	TGAAGTTCGGAATCCCTGATAG	AGGTTCTACTGAGCTATCAGG	
B0868B05-A1.x	BACend	AGCAGAAGAGCAGACCCCTTCAA	GGAGCATCCAATCTTTGAAGGG	
B0598D10-A1.y	BACend	AGATGCTTATACTTGGTGAAG	TACTTACACAGTTGCTTACACC	
B0835H04-A1.y	BACend	AGTCACACCTATGAGGCATCA	CTGTATGAATCCTCTGATGCCT	
B0868B20-A1.y	BACend	ATCCTGCTTTGGGTAGCCAC	AATGCCACGGTGCAGTGGCTAC	
B0700H07-A1.y	BACend	ACTCAACCAACCTTCCATTCA	GGTAGGATTAGTGAATGGA	
B0726A20-A1.y	BACend	TCAGTTCTCAGTCCCTAGGAGAC	GGTCTTCTACTCCAGTCTCCTA	
B0761L21-A1.y	BACend	GGGAGGCTGCTGCTTTCTCA	AAATTAGCCAGGCATGAGAAAG	
B0814G08-A1.y	BACend	GCAGAGGGTGGTGAATGCATC	TGACAGTTTCTCTTTGATGCAC	
B0857A05-A1.y	BACend	TGCTTATCAAGATGCCCTTGCC	AATCAGGCCATGAGGGCAAAGG	
B0895C23-A1.y	BACend	CCATCCTTCATCCCCAGCAGTA	CCCTGAATTTAGGTTACTGCTG	
B0831G12-A1.y	BACend	AGAACCAGGCAGAGCTACCTGG	CTGGACCAGGAATCCAGGTAG	
B0849E15-A1.y	BACend	ACTAGCTATTGAAGTGAATATC	ATGGGCAAGAAATAGATAGTCA	
B0804M18-A1.y	BACend	GTTTCAAGCTGGAAATGTTA	TGCTTCTCTCCCTTAAACATTT	
B0833K01-A1.y	BACend	ATGCTGCTTCATATACACATT	CGGGAAGCATTTGCAATGTGT	
B0863H23-A1.y	BACend	CTCGCTCCATCTGCGATGCACA	AGGTGATCAGAGACTGTGCATC	
B0898L08-A1.y	BACend	TGTTGTGTCAGAAACTCAGGAA	ACCCAGCTGAATCCTTCTGAG	

B0702C13-A1.y	BACend		TGCTGGGGTGGCTTTGACCTTG	TGGCTCAAAGGCTCAAGGTCA
B0702F18-A1.y	BACend		CATGGTCACCTGCAGCCTCTCA	TGGCTAGAAGGAGGTGAGAGGC
B0738O20-A1.y	BACend		AGAAGCGGGGTGAGCAGACAT	GTTACCCGGGAGTTATGTCCTG
B086B05-A1.y	BACend		GATGTTGTCCGACAGGCATGGG	TTCTGTGTAGATCCCCATGCC
B0883G23-A1.y	BACend		GTGGTAGAATTGGCAAGCCTTG	CTCCAATCAGTTGCCAAGGCTT
B0908L18-A1.y	BACend		GGTAAGGACACCTTCAAGGGAC	TGGAGTGCCCTGTTGTCCCTTG
B0974M10-A1.x	BACend		ATGCAAAAGGTCTCAGGACGAAA	CCCTTCCTGGACAATTCGTCC
B1118L08-A1.x	BACend		GGCATGTAGATCAAAATGAATA	TGCTCTAGCTGAATATTTCAT
B0723P10-A1.x	BACend		GGTAGCAGCTTACACTGCTGG	CCTTTCCGATGACCCAGCAGT
B0748H08-A1.x	BACend		TGCCATGTAACGTTCAATTC	GTTTTCCTGTGCAGGGAATATG
B0825F09-A1.x	BACend		ATACCCACAGGGTAGTAACAGT	TTGTGGCTCAAAATCAGCTTTAC
B0825K21-A1.x	BACend		CGTAGGCCCATTTCAACCACAC	TCCCTGTCTTTGAAGTGTGTT
B0845N16-A1.x	BACend		ACATATGAAAAGACCGTAGAAA	CAATTCACAGGCACCTTCTACG
B0894N08-A1.x	BACend		ACGTGGAGAAGGCGCTGCTT	CTGGACATTGAATAAAGACAGC
B0956I11-A1.x	BACend		TGAATTTAACAGGTGGCAAG	ATCCATCTGCACAGCTTTGCCA
B0974M10-A1.y	BACend		CTCATAGTTGTACACACTCTG	AAGCACGTGTTGAACAGAGTGT
B0846E20-A1.y	BACend		CTCCATAGGAAGCAGCCATCAG	ACTGGACCCAGCAACTGATGGC
B0723P10-A1.y	BACend		TGTACCAAACTGTTGACTATTA	GTTTGCCTCATGCTTAATAGTC
B0748H08-A1.y	BACend		GCCTGCACAGGACACAATTGCA	TTCCGGGTTTGATGTGCAATTG
B0825K21-A1.y	BACend		CAATAATTAGTTCCAAATGGCGC	CACAGTCAGAGTTGGCGCCATT
B0845N16-A1.y	BACend		GAGTGCTCACCGGAAGAGAAGA	TCCAGAGCCAACTGTCTTCTCT
B0894N08-A1.y	BACend		TGCCCTTCTTCCTTAGAGCTCC	CATCTGGATTAGCTGGAGCTCT
B0958I11-A1.y	BACend		TGTGGGATGCTTCCAGTTTTGT	GATGAGTAGATCCCAACAAACT
B0861F22-A1.x	BACend		CATCCTGCCTCGGCTCTGAAC	GGTCACTGCAGGAAAGTTTCAGA
B0588P16-A1.x	BACend		AAGAAGGACCTCAACCAAGAGC	ACCCATGTGTGTGCTAGCTCTTG
B1000B21-A1.x	BACend		TATTACAGAGGCTGGTGATCAG	TAGCCTGTGACAGGCTGATCAC
B0839D11-A1.x	BACend		GACAACCTGCTTCTTTTACCTG	AGATGACCTATTGCCAGGTAAA
B1052D15-A1.x	BACend		CAGAAGCATAGAACAAATCCAG	GCACGTGTTTATACGTGGATTG
B1093F08-A1.x	BACend		TGCTGCAACTGCCAAAGAAATTC	CCCTGGCGTTGCAGGAATTCCT

B1134M23-A1.x	BACend		GAATGGGGAGAAAGGGCAAAGG	GCTCGTTAAGAGTTCCTTTGCC
B0894M06-A1.x	BACend		TCTTTCATCTCTAATGGGCAC	TGGGTACATGCACCTGTGCCAT
B0895J20-A1.x	BACend		ACAGACACCTTGGGTCAATGACT	GGAACTGGATGTAAGTCAATGA
B0891F22-A1.y	BACend		CAGTGGTCCCTCTCTCATGAGT	CTGCTTCTAGAACAACTCATGA
B0688P23-A1.y	BACend		ACATGATGCACCCCTTACCGTT	CGGTCTGTGTCAGAACCGGTAA
B0598P16-A1.y	BACend		ACATGGGCTCACAGGAAGATCT	CAGGACTTAGGAGGAGATCTTC
B1000B21-A1.y	BACend		AAGAGAAAGTCGGAGACTGTGTC	TAGCAAGTCTTATCGACACAGT
B0839D11-A1.y	BACend		CCACTCAACCCACAATCTAGTC	GAATACAGGGATGGGACTAGAT
B1052D15-A1.y	BACend		CCACCAATGGATCTGTGACT	ATCAGAGGCTGTAAAGTCAACA
B1093F08-A1.y	BACend		AGCCGGTTTCTTACTACAGAA	TCGAAACAGCTGCCCTTCTGTAG
B1134M23-A1.y	BACend		ACAGAAAGCCGCGGGTAGAGA	TTCTCCATTACGCTCTCTACC
B0894M06-A1.y	BACend		CACATCGCTGCTTGACAGAACT	GGGTATGTGACTGAGTCTCTGT
B0895J20-A1.y	BACend		CACATTTCTGAGACACTTGCTA	TAATACCTGGCATGTAGCAAGT
B0604N13-A1.x	BACend		ATGAGTCTCTCACCGAATGTG	GAACCTCAGTCTCTGCACATTGG
B0714L01-A1.x	BACend		TCATCAGTTCTAGGAGCTTCA	GTAAGTACTCTCTCTGAAAAGCT
B0754A14-A1.x	BACend		GGATCGCACAGTCACTCTTCAT	TGCAAGCGGATATGATGAAGAG
B0894M06-A1.x	BACend		GATTAGTGATGGTAGAGGACA	TGGTGCAGGATTGTTGTCTCT
B1128L12-A1.x	BACend		TTGGTGTGAATCAAGCATCAGG	TGAGCACAGGAGTTCCTGATGC
B0643F18-A1.y	BACend		GTGGATTAAACCGAGGTGGAAT	CCTTTCCAGTTTGAATTCACCC
B0714L01-A1.y	BACend		GGCATTCTTGTGCTGCTCTCTG	GAATACTGCAGAGCAGCAAGCA
B0754A14-A1.y	BACend		ATCCTGGGCAAGGGAGTTTCAG	CTGAGCCACACCTTCTGAAACT
B0894M06-A1.y	BACend		TTGTTACATCGCTGCTTGACA	ATGTGACTGAGTCTGTCAAGC
B1128L12-A1.y	BACend		GCTTGAACCTGCACCTCAGCAGGA	GTGCTTCTAACTTCTCTCTGCTG
B0687F10-A1.x	BACend		TCTCTCAAGCCACTTCTATGT	ACGTGAATCACGGGAACATAGAA
B0791C09-A1.x	BACend		ACTGTGGCTGCACATAGGGATA	AAAGCTTCTGGGGTATCCCTA
B0820N18-A1.x	BACend		GGACCCACCCCTGTCAATTTTCAT	GGGCGGATGGGAATATGAAATT
B0880M22-A1.x	BACend		TGTTTGGATATGTTGGCTACTA	TGTGTGTTTTGAGTTAGTAGCC
B1008L21-A1.x	BACend		ATCTCTGGGAAGCTCTACAGTG	CTCAAAATCCCTCCCACTGTAG
B1043N20-A1.x	BACend		AGATAATGGGTTGCTTGGGCTC	GTTAAGCAGTTATGAGCCCAA

B0700H07-A2.x	BACend	CTGGACTCAAGACATCCTCTG	TGGGAGACTGAGACCAGAGGAT	
B0687F10-A1.y	BACend	TTTCAGTGAAGTCTCTCCGTT	TGGCTGTAAGTGAACGGAAG	
B0781C08-A1.y	BACend	CATTAGAAGCCAGGAGGAAC	CTCCTTCTCCCGAGTTCTCTC	
B0680M22-A1.y	BACend	CTATGTTGCATAGGAGTAGTGA	AAGGATACCCTCTCTCACTACT	
B0609E24-A1.y	BACend	CCCTCTATAACATTTTCTCCCA	CTTAGGACAACCCCTGGGAGAA	
B1008L21-A1.y	BACend	GAGCCCTGCTCAGAAATTCATG	GAGGCAAGGCTTTTCATGAAT	
B0923H14-A1.y	BACend	GGAGCCTTACTGAGCTGACAGT	CCGTCCATGGGAACACTGTGAC	
B0879G13-A1.y	BACend	CTCCACCTGGATGGGTCAACTT	ATTAAGTTCCTTGAAAGTTGAC	
B1020H18-A1.y	BACend	CATGATCTCAATAATTGCAACT	GAAGAAACAGGAGAGTTGCAA	
B0758E08-A1.y	BACend	ATGGGTATCACTATGCATAGCA	TTTAAATTCACACTTGTATGC	
B0686F01-A1.y	BACend	GTGCTCTGGTGAACGGCTCTGA	AATCAGAGTTTCTTCAGAGCC	
B0883G19-A1.y	BACend	ACATTCCAGCTCTACATCTA	CTGAGTTTCTCAGTAGAATGT	
B0923H14-A1.x	BACend	GATTAAGAGAGGGTAGGAGGGT	ACCTTCAACCATCACCCCTCCT	
B0781H18-A1.x	BACend	GGATTAATAGTACCACCCCTG	ATTTAACACAAAGGCGAGGGGT	
B0979G13-A1.x	BACend	GACATTCATGCAATGGACAC	CCGCTTGGCTTTGGTGTCCAT	
B1020H18-A1.x	BACend	CATATGGCTAAGGCTCTATCTA	AATCAGCAGGTACATAGATAGA	
B1029H23-A1.x	BACend	CAGCTAGGGGAAGAGTGACAGG	CGAAATGCCGACTGCCGTGTAC	
B1078C21-A1.x	BACend	CTAGAAATTCATGTAGTAAGA	ATACTGCTCTTTCTCTTACTA	
B1104N09-A1.x	BACend	CCTGCCTGATGAGCAAGAATA	CACCTGGGTACTTCTTATCTTT	
B0683J16-A1.x	BACend	CAACCAACTATCTGCTGCCTTC	TAGGTGAGTCTCTTGAAGGCGAG	
B0658F13-A1.x	BACend	GGTGTGGAGAGAGTGGACTCTA	TAATATAAATCCTTAGAGTCC	
B0883G19-A1.x	BACend	CATGGCACAGGTGATAGAGTGA	ATAATCCAGGAAGATCACTCTA	
B0760A04-A2.x	BACend	GCCTCATGATTGGGCAATGCT	GTTCAAAATCTTGAAGCATGCC	
B0785D22-A1.x	BACend	GTGAACAGGCTAACACTGTAA	ATGCGTGGTGGTGTAAACAGT	
B0723P10-A1.y	BACend	TGGAAGCCACTTAGAGGTTGCA	AACAGTTTGGTACATGCAACCT	
B1095L07-A1.x	BACend	TCTAAAGATGGGGCTCACAGT	ATGGCTTCAGTTTTACTGTGAG	
B0997H04-A1.x	BACend	TACTTACTCTGTTTCTCTGTAT	AAGTGATATGAGACATACAGGA	
B0723P10-A1.x	BACend	AGGAAGGGAAATAGAGGGAA	TATCTGCGTGGTGGTTCCTTTC	
B0997H04-A1.y	BACend	AGTGTAGTGGGAATGAGGAGT	CTCCATTATCAGTCACTCCTCA	

B0880L16-A2.x	BACend		GAAACCCACATCAGCACAAAGG	TTGTGCTGGCTGGCCTTTGTG	
B0588O21-A2.x	BACend		CGCCGAATTCCATGACTCTGA	TTTGGCAGAATGTTTCAAGAGT	
B0768I12-A2.x	BACend		CACAAAGACAGACCCACAGCTC	GCTGTGGAAATGTGAGCTGTG	
B1056C02-A2.x	BACend		CCACACAGGAAACTGCCATCT	CCAACTCTCTTCAGATGGCA	
B1056C02-A2.y	BACend		GAGACGTGAGTCAGGACAGGTG	TGCCAATCTGTACCACCTGTC	
sIs-AA017225	EST		GATGCCAGGAAGTACCTGGTAA	GCAATCTCCAATCCTTACCAGG	
A004F14	EST		GGAAACCCGTGACTTGACTTAG	TGTCATCAGCACCCCTTAAGTCA	
SGC31333	EST		AGGTGTGATCTAGTCTCCGGT	GAGTGAAGGTGGAACCGGAGA	
WI-12422	EST		AACCAGACAGCATCTCTGGAGAGA	CACAGAGGTGCATTTCCTCCA	
sISG21539	EST		ATGCATACAGCAGGCCATTGTG	CAGCCCCCTATGACCACAATGG	
WI-13120	EST		GGGAGCTACAGGTGATAGTAT	GGCGCATAGCTATCACCTGTA	
sISG22703	EST		CACCAGAGACCAGAGACTCGAA	ACCATGGACAGGCTTCGAGTC	
sISG36087	EST		TGAGCAGTCTGACCTGCTTCTC	AGCTGGAGCACCTGGAGAAGCA	
sISG8807	EST		CAGCCAGCTACTGAACCTTATG	TGCCCTAGGCACACATAAGGT	
sISG15434	EST		TACCACACCCCTGCCAGATGG	GTANTCTGTGGCGCCCATCTGC	
sISG30525	EST		GGCACACAGTCTGCAATGCTTG	TAGGGACATCCCTCAAGCATT	
A007A34	EST		TGTTCTGGCAGATTCCATCATC	CTTATGTTGGGATTGATGATGG	
A006D44	EST		CAGGGTCAATTGGAGGGAACA	CGAAAGCTTGAATCTGTTCCCTC	
SGC30248	EST		GATGCAAGCAGCACAGAGCAGT	CTCCTTCCACAGCACTGCTCT	
sIs-N20163	EST		TCTCTACCAGGCAATACTTCAC	CTGAAATCGAGTGAGTGAAGTA	
Cda0aI01	EST		AAAGGCCACACAGCCCAATC	GGCCTGCAGTGGATGATTGTGG	
Cda0ca07	EST		AAGTCTGACTTCAATCGGTAC	TGCTAAGCCTCATGTACCGAT	
sISG3292	EST		AAGTCTGACTTCAATCGGTAC	TGCTAAGCCTCATGTACCGAT	
SGC34088	EST		AAGTCAATTGCTCCCATCTGC	CTTGTTCGTGCTGGCAGATGG	
WI-12272	EST		GACTCATATGACAGACCTTGA	TGCCCACTTTCCTTCAAGGT	
sISG16387	EST		CATGACTCCCAGACCCCTTGA	TGCCAAATTCCTGTCTAAGGG	
SGC31722	EST		CAAAACGGAGAAGCCCCAGATAC	TTGTTACTGTAGCTGTATCTGG	
WI-15018	EST		AGTGACAATTAGAGCTCTGGGG	GCTCCTTCATCTCCCCCAGAG	
WI-18482	EST		TGCTTGGCCAACACAGACTTCCT	TGATGAGACTGCAGAGGAAGTC	

sISG36546		EST	ACCTGAGAGCAGGAGATTCCA	TAATCCTAGCAGCTGGAACTCT
A06016		EST	CCCGAGGCTTCTCTGAACACTA	CTCAGAGCGCTTTCTAGTGTTCT
H64839		EST	AATCTGAGGCACACAGGAGAGT	ACTGAGCTCCTTTCTACTCTCCT
sISG3357		EST	GCCTTGCTAACTGTACCATAGT	CACCTGCAGGAATAACTATGGT
sISG30906		EST	TCTAAGGTTCCGGATGGACGTG	TGTCCCGCCAAATTCAGTCCA
sISG28056		EST	GAGTTACAGGAAGTGGTTCGCC	CTGCGTGTCTGTCAAGGGGAACC
SGC30786		EST	ACAGCTCTCCTTCTCTTAATGCC	CACCCCTTATCTCTGGGCATTAA
sIs-N59820		EST	AGACTGCATCTCTCGAACACAGG	ACTGGAAATCTAGCGCCTGTTGT
sISG42115		EST	TTCTCGAGGGTTCCTCTGCTTCACT	AGTTCTCTCGGGAGTTAGTGAAGC
FB9F8		EST	GAAAAACCGGCACCGCTGACACAAC	CGTCCAGAAAACGTAAGTTGTGTC
AA252357		EST	CAGCACATCGAGTCTCTCAATCCG	CCAGACTTTCCTCAGCTCGGATTG
sISG4720		EST	TCGAGAAAGGCTGTCTCTACAAGG	TAACTCAGGACCTTCCCTTGTAG
sIs-AA001424		EST	AAGCTGCTCTTCTCAGCTACTCTG	TTTCAGGGTTCTGGGTGAGAGTAG
sISG31443		EST	CAAAGCACTGGACTGAGAGAATTC	GGTGGATACAGTGTGTGAATTTCTC
WI-6385	D12S1405	EST	TAAAGGCAAGGCGCACACAGCCCA	CTGCAGTGGATGATTGTGGGCTGT
A008Y05		EST	TAAAGATAAGGCGTGGCTTTGAC	AACTCTGGCAGACACTGTCAAAGC
R50113		EST	TCATACCAGTGCTGGCTGCTAAG	CCAGTTTCTCCACATCCTTAGCAG
sIs-H94885		EST	CTCTAAGAACCCAGACCCCTCAGTTG	CTCATTCCTTACTGGCACTGAG
A006R19		EST	GGTTTGAACAGTGGGAGATACCAG	TTTTCTCTCCACCTCTGGTATC
SGC34278		EST	CAACACAAAGAGGTCCTCTTGCTG	ACAGTCCATGGAAGGCGAGCAAGA
A004B47		EST	GTGCCCTGTGAATTGGCCTTTCT	GCTGGAAGCAGAAAAGAAAGGCG
sISG40199		EST	GGAAGGCTGTCTCTTTCTTACCAC	TGACACCTGCTCATGGTGGTAGA
sISG8935		EST	CAAAACAAAGAGGTCTCTTGCTG	ACAGTCCATGGAAGGCGAGCAAGA
sISG4731		EST	GCATGTGTGTTTCTGTCTGGGAT	AGCAGACAAGATCTAGATCCCAGA
sISG8142		EST	GTGCCCTGTGAATTGGCCTTTCT	GCTGGAAGCAGAAAAGAAAGGCG
A005X42		EST	GCATGTGTGTTTCTGTCTGGGAT	AGCAGACAAGATCTAGATCCCAGA
CDA18G06	D12S1205E	EST	ACAGACTACAAGTCAATGAAGCC	AGCAGACAAGATCTAGATCCCAGA
STSG40222		EST	TCCTCTCTCTACTGCAGACCATG	TCCGACAATGCCAGGAGGCTTCAT
sIs-R55615		EST	GCTAGTGAACGGGATACCTGAAG	TGCCACATGGAGAAAACATGGTCT
		EST		CTTCTGTGGTAGTGTCTTTTCAGG

s1s-R02285	EST		CTCAATCCACATGACAAGGCTTTG	ACCTAGTATCTACTCAAGGGT	
s1s-R81342	EST		GGCAAAAGGGAACCAACCATGATG	TCACCTCCCTTACAGTCATACATG	
s1s-H65839	EST		AATAGATTGATGCCGTCCCTCAAC	AAGTATGTGCTAACTTGTGAGGA	
s1sG52716	EST		AGATGGGGGAGACAACGGAAC	CGGAAAGGAACATCTGTTACCG	
s1sG54813	EST	highly similar to 22 kd peroxisomal membrane protein	TTTGTGGTCAGCTGGTCCAACCA	TGCAGTAATGATGGGTGGTTGGA	
s1sG50504	EST		CCGTATTACCCAGACTACACACTG	CACCAATGGCATAGCACAGTGTGT	
s1sG48386	EST		CCAGCAGCAGGATATTGTAGGT	GTTTACAGCCTACAGGACGTACAC	
s1sG54842	EST		TTCTTCTTCAGTCCGCTCAAAG	TCAGGGCTACGAGATCTTTGAGC	
s1sG53860	EST	Highly similar to peptidase transporter PTR2	AACTGGGATGCCAACTAACACGTG	AAGTCTTGGGGAACCTCCACGTGT	
s1sG53541	EST	Homo sapiens hwi mRNA, partial cds	AACCCACCTATGGTTGTAGTGAG	GGCGTAAAGTAGGATGCTCACTAC	
s1sG53307	EST		GAGGCTAGGCTGAATATTAACAGG	CACCTGCCAGTCAGCAACCTGGTTA	
s1sG83473	EST		CCACTGGCTGCATTTTCCAGCTTT	CACCAGGTACTAGAGAAAAGCTGG	
s1sG54325	EST		CGGCACAAGCAGATTTCAGATCAG	CTGGGGAAATGCTGACTGATCTG	
s1sG52343	EST		AACTGGAGTCAGGTGATCAGGAAG	CCAGTGAATAAGCCCCCTTCGTGA	
WIAF-856	EST		AAGTCAATTGCTCCCCATCTGCCA	TCTACTTGTTCGTTGCTGGCAGAT	
s1sG47723	EST		CTGAGTTCTTAGCAGCTTCCGTA	TCTTCAAAGGAGCTCCTACGGAAG	
s1sG80065	EST		GGAGGTGAATAAGCTGATCCTGCA	GCTGGGTAACTAGAAGTGCAGGAT	
s1sG48424	EST		GGACACATCTGTTCCATCTCACC	CCCATGAGTTGTTAGTGGTGAAGA	
s1s-LJ79526	Gene	DEZ	TGATCCTCACTGTGGAACCCCT	GAGAGAGTCCATTGAGGGGTTTC	
SGC31491	Gene	NOS1	AGAGCGGCTCTTTTAATGAGGG	GGGAGACGTGCGCAACCCCTCATT	
s1sG1938	Gene	CLA-1	TCAGTCCATAGGATGATGTCAG	TCCTCCAGCTAAACTGACATC	
s1s-W31616	Gene	UBA52	CCACGAAAGATCAACCTCTGC	ATCCTCTCTGATCAGCAGAGGT	
ZNF10	Gene	KOX1	ATGTGGGAAGGCCCTTTGGTAGT	GTAAGGTTTGAGCCACTACCAG	
ZNF26	Gene	KOX20	GTGAATGTGGAAGGCCCTTCAC	GAGATGACTTCTGAGTGAAGGC	
WI-6921	Gene	RNP24	GTTGCAAGTGTCTCACCCTAAG	AACCATCTTCCACCTTGGGTG	
s1s-D60472	Gene	SMRT	GAACGACGTGTGTAATGACAG	AGGGTGGTGGTATCTGTCAAT	
WI-16177	Gene	RAN	CCTTACGATCCACACAGATGA	CGGAACATGTGCCCTTCATCTGT	

sISG1702	Gene	CAGH32	TCAGGCACCAATCTGAACAAGGG	GAAGTTGGATCCAAGCCCTTGTT	
IB2452	Gene	ULK1	GCATCAAGGTGATGAGGAAGAAG	AAGAAATCCCGTGACTTCTCC	
sISG39493	Gene	CAGH32	GTGCTGAATCTCTTCGCTGACATG	TAGTGAACCTTGGGACCATGTCAC	
A002A44	Gene	CAGH32	TGGTCTCTGCTTCACTGGCAGAA	GGATAAGCTTGTGTGTTCTGCGCA	
sISG27206	Gene	GCP170	GAGCACATCTGGCCCTGGCCAGT	TGAGGTTCTGAGTCACCTGGCCA	
CD41JF08	Gene	GCP170	AGTGAGCTCAGAACAACCTCACACC	AGTTGAGTGACGCTGTGGTGTGAG	
R39596	Gene	GCP170	ACTTCTGCAGTCATCGAAGATCC	CCCACAAAAGATCCCAGGACTTCT	
sISG31494	Gene	ZNF140	TCTCCAGTATGAGTCTCTGTTGT	GCTTTCCCTGGTGTACACAGA	
TH_a	Gene	MUC8	ATCCACCGCTAGAAACCCACTC	GACCATCAACTGATGAGTGGGT	
SGC31491_a	Gene	NOS1	CCTAGTAGCTTCTCTCCAAAG	ATTGGAAGAAAGCCCTTGGGA	
sIs-X88576	Gene	MMP17	AGAGGAGCTGTCTAAGGCCATC	TGCTGCATGGCTGTGATGGCCT	
sISG43910	Gene	SFRS8	caglaacalgtllacccacagac	tgacacalaegtgcagacacac	
P698K7/77	Genomic		AGAAAGCTCTCTTCCCCTCTCTC	GTCACATTTTGGGGTGAGAGAGG	
P493P14/77	Genomic		TCTCAGAACCCAGAGTCCATAG	CAGTTAGATAAAAGCTATGCAC	
P313C9/SP6	Genomic		CAGCTCAGGAAGTTCAACCCAGGC	AGGACCCAGTTGAAGCCCTGGTG	
WI-5824	Genomic		CATTTACCTGCCCGCTGGTCA	CAGGATTTGTGTGGTGACCAGG	
WI-10803	Genomic		CTGGATTTCCAGAGACTGACCT	TCAGGCAATAGAGAAGGTGAGT	
WI-2002	Genomic		ACAACAGAAAGTTGTCAGTGAAG	CTGTTCAACAGTGCCTTTCACCTG	
WI-3045	Genomic		CTTAAGCGAGAACCTGATAACCC	TCCTAATCTGGCAGGTGGGTATC	
WI-3549	Genomic		GAGAATCAGCTGCCATGTTGTGAG	GGACTCTTGAGCATCCTCACAAC	
WI-6077	Genomic		AGCAGCACTAGGCATGGCTGTT	ATAAGAGCTGAGATAACAGCCA	
SHGC-12243	Genomic		CAAGCTTCCCTCTTCCCATTTGT	TTCCGGCGTTGTAGTTACAAATGGG	
SHGC-13782	Genomic		AGTCAGGTACAGGGTTCTGACAAC	CACCTTGTTCGCTCTGTGTTGCAG	
SHGC-14238_a	Genomic		CAAGTGTCCCACTTTTCTGCA	CCGCTCAGTCACCTGCGAGGA	
WI-3549_a	Genomic		CCATGTTGTGAGGATGCTCAAA	ACCTTTTAGGACTCTTTGAGCA	
AFMb337xc1	MSAT		GATCTGCAGCATGAGGGAGCA	GTCTCTAGGCACATTGCTCCCT	
AFMa197zdf9	MSAT		GGGATTTAGTAGTCAATGTA	GTCATCGGGTGACATACATTGA	
AFMb350zb5	MSAT		GTTTGTAGGCTTCTGCCCTCTG	CCCTCTACCAATTCACAGAGGCA	
UT7009	MSAT		GTCCAAGAGTGGCAGTTGACC	ATTGGATAGGCATAGGTCAACT	

AFMb301we5	D12S1659	MSAT	TCTAACTTTCTGTTTGCCTGCTT	CACTGTGCTTTTCAAGAAGCAGGC
AFMa084xg9	D12S1714	MSAT	GTTCCGAGATCCACAGGTGCTCTA	TGTAGCATATGATGTAGACACC
CHLC.ATA19A08	D12S2069	MSAT	TGTTGCCTAGGCTGGTCTTGA	CTTGAGTCCAAGAGTTCAAGAC
ATA29A08	D12S1045	MSAT	GACCAGCCTAGGCACATAGTGA	TTAGAGATGGGGTCTCACTATG
AFM210zd6	D12S97	MSAT	AATTGTCTCCATGGGCTCGAA	CCTTCACGTGAGGAGTTCGAGCC
AFM285ye8	D12S343	MSAT	TACTGCCACTCTCCAAATATC	GATCTGGAAGGTCGGATATTCT
509/510	D12S63	MSAT	GTGGTTGGTTAACAAGAATG	GAGAAGCTGCAACGGCATCTTT
AFMa275xb9	D12S1628	MSAT	AAGGTAGAGCTTGGCAACAGGA	AGCCCGCTGGACCTCCTGTGTG
AFMb002vd5	D12S1638	MSAT	TGCCAGGAGTTTTAAGTTGGTT	GAATGGCATTGTTGTAACCAACT
GATA13D05	D12S362	MSAT	GTATGGATAGCAGACGATAGAG	TCTATCTGTCACTCCCTCTATCG
120TEL82	D12S2342	MSAT	TACATTCCACAGCAGTGCACAAG	TGGAGAAATTGGAAGCCTTGTCGA
120TEL87	D12S2343	MSAT	TGTTAGGCTTCTGGGTGGGTAC	ACAGGCATTAGCCGCTGTACCGAA
AFMa082ze9_a	D12S1723	MSAT	CTTCCGTCATGAATGTCAGTAG	TCTGCAGTGGTTCCTACTACTGAC
AFM156xc5_a	D12S1599	MSAT	TGGGAAGAGTTGCCTCCAGGAA	CCCTTCTCAGTCTTTTCTCTGGA
AFMa123ve1	D12S367	MSAT	CTGTATTAATGAGTCTGGGTT	GGGTTAATACAGTTAACCCAGA

3 Radiation Hybrid (RH) Mapping: Radiation hybrid mapping was performed against the Genebridge4 panel (Gyapay et al., 1996, *Hum. Mol. Genet.* 5:339-46) purchased from Research Genetics. Mapping was performed in order to refine the chromosomal localization of genetic markers used in genotyping as well as to identify, confirm, and refine localizations of markers from proprietary sequences. Standard PCR procedures were used for typing the RH panel with markers of interest.

10 Briefly, 10 µl PCR reactions contained 25 ng DNA of each of the 93 Genebridge4 RH samples. PCR products were electrophoresed on 2% agarose gels (Sigma) containing 0.5 µg/ml ethidium bromide in 1 X TBE at 150 volts for 45 min. Model A3-1 electrophoresis systems were used (Owl Scientific Products, Portsmouth, NH). Typically, gels contained 10 tiers of lanes with 50 wells/tier. Molecular weight markers (100 bp ladder, GibcoBRL, Rockville, MD) were loaded at both ends of the gel.

15 Images of the gels were captured with a Kodak DC40 CCD camera and processed with Kodak 1D software (www.kodak.com). The gel data were exported as tab delimited text files. The names of the files included information about the panel screened, the gel image files, and the marker screened. These data were automatically imported using a customized Perl script into Filemaker databases for data storage and analysis. The data were then automatically formatted and submitted to an internal server for linkage analysis to create a radiation hybrid map using RHMAPPER (L. Stein et al., 1995; available from Whitehead Institute/MIT Center for Genome Research, at <http://www.genome.wi.mit.edu/ftp/pub/software/rhmapper/>, and via anonymous ftp to ftp.genome.wi.mit.edu, in the directory /pub/software/rhmapper.)

30 4. BAC Library Screening: The protocol used for BAC library screening was based on the "overgo" method, originally developed by John McPherson at Washington University in St. Louis (<http://www.tree.caltech.edu/protocols/overgo.html>, and W-W. Cai et al., 1998, *Genomics* 54:387-397). This method involved filling in the overhangs generated after

annealing two primers. Each primer was 22 nucleotides in length, and overlapped by 8 nucleotides. The resulting labeled product (36 bp) was then used in hybridization-based screening of high density grids derived from the RPCI-11 BAC library (deJong, *supra*). Typically, 15 probes were
 5 pooled together to hybridize 12 filters (13.5 genome equivalents).

Stock solutions (2 μ M) of combined complementary oligos were heated at 80°C for 5 min, placed at 37°C for 10 min, and then stored on ice. Labeling reactions included the following: 1.0 μ l H₂O; 5 μ l mixed oligos (2 μ M each); 0.5 μ l BSA (2 mg/ml); 2 μ l OLB (-A, -C, -N6) Solution (see
 10 below); 0.5 μ l ³²P-dATP (3000 Ci/mmol); 0.5 μ l ³²P-dCTP (3000 Ci/mmol); and 0.5 μ l Klenow fragment (5 U/ μ l). The reaction was incubated at RT for 1 hr, and unincorporated nucleotides were removed using Sephadex G50 spin columns. Solution O: 1.25 M Tris-HCl, pH 8, and 125 M MgCl₂; Solution A: 1 ml Solution O, 18 μ l 2-mercaptoethanol, 5 μ l 0.1M dTTP, and 5 μ l 0.1 M
 15 dGTP; Solution B: 2 M HEPES-NaOH, pH 6.6; Solution C: 3 mM Tris-HCl, pH 7.4, and 0.2 mM EDTA; Solutions A, B, and C were combined to a final ratio of 1:2.5:1.5, and aliquots were stored at -20°C.

High-density BAC library membranes were pre-wetted in 2 X SSC at 58°C. Filters were then drained slightly and placed in hybridization solution
 20 (1% BSA; 1 mM EDTA, pH 8.0; 7% SDS; and 0.5 M sodium phosphate), pre-warmed to 58°C, and incubated at 58°C for 2-4 hr. Typically, 6 filters were hybridized in each container. Ten milliliters of pre-hybridization solution was removed, combined with the denatured overgo probes, and added back to the filters. Hybridization was performed overnight at 58°C.
 25 The hybridization solution was removed and filters were washed once in 2 X SSC, 0.1% SDS, followed by a 30 min wash in the same solution at 58°C. Filters were then washed in: 1) 1.5 X SSC and 0.1% SDS at 58°C for 30 min; 2) 0.5 X SSC and 0.1% SDS at 58°C for 30 min; and in 3) 0.1 X SSC and 0.1% SDS at 58°C for 30 min. Filters were then wrapped in Saran
 30 Wrap®, and exposed to film overnight. To remove bound probe, filters were treated in 0.1 X SSC and 0.1% SDS pre-warmed to 95°C, and then cooled

to RT. Clone addresses were determined in accordance with instructions supplied by RPCI.

To recover clonal BAC cultures from the library, a sample from the appropriate library well was plated by streaking onto LB agar (T. Maniatis et al., 1982, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) containing 12.5 µg/ml chloramphenicol (Sigma), and plates were incubated overnight. A single colony and a portion of the initial streak quadrant were inoculated into in each well of a 96-well plate containing 400 µl LB plus chloramphenicol. Cultures were grown overnight at 37°C. For storage, 100 µl of 80% glycerol was added to each well, and the plates were placed at -80°C.

To determine the marker content of clones, aliquots of the 96-well plate cultures were transferred to the surface of nylon filters (GeneScreen Plus, NEN) placed on LB/chloramphenicol petri plates. Colonies were grown overnight at 37°C and colony lysis was performed by placing filters on pools of: 1) 10% SDS for 3 min; 2) 0.5 N NaOH and 1.5 M NaCl for 5 min; and 3) 0.5 M Tris-HCl, pH 7.5, and 1 M NaCl for 5 min. Filters were then air-dried and washed free of debris in 2 X SSC for 1 hr. The filters were air-dried for at least 1 hr, and DNA was crosslinked linked to the membrane using standard conditions. Probe hybridization and filter washing were performed as described above for the primary library screening. Confirmed clones were stored in LB containing 15% glycerol.

In certain cases, polymerase chain reaction (PCR) was used to confirm the marker content of clones. PCR conditions for each primer pair were optimized with respect to MgCl₂ concentration. The standard buffer contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, MgCl₂, 0.2 mM each dNTP, 0.2 µM each primer, 2.7 ng/µl human DNA, 0.25 U AmpliTaq (Perkin Elmer) and MgCl₂ concentrations of 1.0 mM, 1.5 mM, 2.0 mM or 2.4 mM. Cycling conditions included an initial denaturation at 94°C for 2 min; 40 cycles at 94°C for 15 sec, 55°C for 25 sec, and 72°C for 25 sec; and a final extension at 72°C for 3 min. Depending on the results, the conditions were further

optimized as required. For further optimization, the annealing temperature was increased to 58°C or 60°C, the cycle number was increased to 42, the annealing and extension times were increased to 30 sec, and/or AmpliTaqGold was used (Perkin Elmer).

- 5 5. BAC DNA Preparation: Several different types of DNA preparation methods were used to isolate BAC DNA. The manual alkaline lysis miniprep protocol listed below (Maniatis et al., 1982) was successfully used for most applications, i.e., restriction mapping, CHEF gel analysis, and FISH mapping, but this protocol was not reproducibly successful for
10 endsequencing. The Autogen protocol described below was used to isolate BAC DNA for endsequencing.

- For manual alkaline lysis BAC minipreps, bacteria were grown in 15 ml terrific broth (TB) containing 12.5 µg/ml chloramphenicol. Cultures were placed in a 50 ml conical tube at 37°C for 20 hr with shaking at 300 rpm.
15 Cultures were centrifuged in a Sorvall RT 6000 D at 3000 rpm (1800 x g) at 4°C for 15 min. The supernatant was aspirated as completely as possible. In some cases, cell pellets were frozen at -20°C at this step for up to 2 weeks. The pellet was then vortexed to homogenize the cells and minimize clumping. Following this, 250 µl of P1 solution (50 mM glucose, 15 mM Tris-HCl, pH 8, 10 mM EDTA, and 100 µg/ml RNase A) was added. The mixture
20 was pipetted up and down to mix. The mixture was then transferred to a 2 ml Eppendorf tube. Subsequently, 350 µl of P2 solution (0.2 N NaOH, 1% SDS) was added, mixed gently, and the mixture was incubated for 5 min at RT. Then, 350 µl of P3 solution (3 M KOAc, pH 5.5) was added and mixed
25 gently until a white precipitate formed. The solution was incubated on ice for 5 min, and then centrifuged at 4°C in a microfuge for 10 min.

- The supernatant was transferred carefully (avoiding the white precipitate) to a fresh 2 ml Eppendorf tube, and 0.9 ml of isopropanol was added. The solution was mixed and left on ice for 5 min. The samples were
30 centrifuged for 10 min, and the supernatant was carefully removed. Pellets were washed in 70% ethanol and air-dried for 5 min. Pellets were then

resuspended in 200 µl of TE8 (10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA, pH 8.0), and RNase (Boehringer Mannheim, <http://biochem.boehringer-mannheim.com>) added to 100 µg/ml. Samples were incubated at 37°C for 30 min, then precipitated by addition of NH₄OAc to 0.5 M and 2 volumes of ethanol. Samples were then centrifuged for 10 min, and the pellets were washed with 70% ethanol. The pellets were air-dried and dissolved in 50 µl TE8. Typical yields for this DNA prep were 3-5 µg per 15 ml bacterial culture. Ten to 15 µl of DNA was used for *EcoRI* restriction analysis; 5 µl was used for *NotI* digestion and clone insert sizing by CHEF gel electrophoresis.

Autogen 740 BAC DNA preparations were made by dispensing 3 ml of LB media containing 12.5 µg/ml of chloramphenicol into autoclaved Autogen tubes. A single tube was used for each clone. For inoculation, glycerol stocks were removed from -70°C storage and placed on dry ice. A small portion of the glycerol stock was removed from the original tube with a sterile toothpick and transferred into the Autogen tube. The toothpick was left in the Autogen tube for at least 2 min before discarding. After inoculation the tubes were covered with tape to ensure that the seal was tight. When all samples were inoculated, the tubes were transferred into an Autogen rack holder and placed into a rotary shaker. Cultures were incubated at 37°C for 16-17 hr at 250 rpm.

Following this, standard conditions for BAC DNA preparation, as defined by the manufacturer, were used to program the Autogen. However, samples were not dissolved in TE8 as part of the program. Instead, DNA pellets were left dry. When the program was completed, the tubes were removed from the output tray and 30 µl of sterile distilled and deionized H₂O was added directly to the bottom of the tube. The tubes were then gently shaken for 2-5 sec and then covered with parafilm and incubated at RT for 1-3 hr. DNA samples were then transferred to an Eppendorf tube and used either directly for sequencing or stored at 4°C for later use.

6. BAC Clone Characterization: DNA samples prepared either by manual alkaline lysis or the Autogen protocol were digested with *EcoRI* for analysis of restriction fragment sizes. These data were used to compare the extent of overlap among clones. Typically 1-2 µg DNA was used for each
5 reaction. Reaction mixtures included: 1 X Buffer 2 (NEB); 0.1 mg/ml BSA (NEB); 50 µg/ml RNase A (Boehringer-Mannheim); and 20 U *EcoRI* (NEB) in a final volume of 25 µl. Digestions were incubated at 37°C for 4-6 hr. BAC DNA was also digested with *NotI* for estimation of insert size by CHEF gel analysis (see below). Reaction conditions were identical to those for the
10 *EcoRI* digestion, except that 20 U *NotI* were used. Six microliters of 6 X Ficoll loading buffer containing bromphenol blue and xylene cyanol was added prior to electrophoresis.

EcoRI digests were analyzed on 0.6% agarose gels (Seakem, FMC Bioproducts, Rockland, ME) in 1 X TBE containing 0.5 µg/ml ethidium
15 bromide. Gels (20 cm x 25 cm) were electrophoresed in a Model A4 electrophoresis unit (Owl Scientific) at 50 volts for 20-24 hr. Molecular weight size markers included undigested lambda DNA, *HindIII* digested lambda DNA, and *HaeIII* digested X174 DNA. Molecular weight markers were heated at 65°C for 2 min prior to loading the gel. Images were
20 captured with a Kodak DC40 CCD camera and analyzed with Kodak 1D software.

NotI digests were analyzed on a CHEF DRII (Bio-Rad) electrophoresis unit according to the manufacturer's recommendations. Briefly, 1% agarose gels (Bio-Rad pulsed field grade) were prepared in 0.5
25 X TBE, equilibrated for 30 min in the electrophoresis unit at 14°C, and electrophoresed at 6 volts/cm for 14 hr with circulation. Switching times were ramped from 10 sec to 20 sec. Gels were stained after electrophoresis in 0.5 µg/ml ethidium bromide. Molecular weight markers included undigested lambda DNA, *HindIII* digested lambda DNA, lambda ladder PFG
30 ladder, and low range PFG marker (all from NEB).

7. BAC Endsequencing: The sequence of BAC insert ends utilized DNA prepared by either of the two methods described above. The ends of BAC clones were sequenced for the purpose of filling gaps in the physical map and for gene discovery information. The following vector primers specific to the BAC vector pBACe3.6 were used to generate endsequence from BAC clones: pBAC 5'-2 (TGT AGG ACT ATA TTG CTC; SEQ ID NO:) and pBAC 3'-1 (CGA CAT TTA GGT GAC ACT; SEQ ID NO:).

The ABI dye-terminator sequencing protocol was used to set up sequencing reactions for 96 clones. The BigDye (ABI; PE Applied Biosystems) Terminator Ready Reaction Mix with AmpliTaq[®] FS, Part number 4303151, was used for sequencing with fluorescently labeled dideoxy nucleotides. A master sequencing mix was prepared for each primer reaction set, and included: 1600 µl of BigDye terminator mix (ABI; PE Applied Biosystems); 800 µl of 5 X CSA buffer (ABI; PE Applied Biosystems); and 800 µl of primer (either pBAC 5'-2 or pBAC 3'-1 at 3.2 µM). The sequencing cocktail was vortexed to ensure it was well-mixed and 32 µl was aliquoted into each PCR tube. Eight microliters of the Autogen DNA for each clone was transferred from the DNA source plate to a corresponding well of the PCR plate. The PCR plates were sealed tightly and centrifuged briefly to collect all the reagents. Cycling conditions were as follows: 1) 95°C for 5 min; 2) 95°C for 30 sec; 3) 50°C for 20 sec; 4) 65°C for 4 min; 5) steps 2 through 4 were repeated 74 times; and 6) samples were stored at 4°C.

At the end of the sequencing reaction, the plates were removed from the thermocycler and centrifuged briefly. Centri-Sep 96 plates were then used according to manufacturer's recommendations to remove unincorporated nucleotides, salts, and excess primers. Each sample was resuspended in 1.5 µl of loading dye, and 1.3 µl of the mixture was loaded onto ABI 377 Fluorescent Sequencers. The resulting end sequences were then used to develop markers to rescreen the BAC library and fill sequence

gaps. The end sequences were also analyzed by BLASTN to identify EST or gene content. The BAC end sequences correspond to SEQ ID NO:156 to SEQ ID NO:693, disclosed herein.

EXAMPLE 5: SUBCLONING AND SEQUENCING OF BACs
FROM 12q23-qter

5 The physical map of the chromosome 12 region provided a set of BAC clones for use as sequencing templates (see Figures 5A-5I). BAC DNA was isolated according by a QIAGEN purification (QIAGEN, Inc., Valencia, CA, per manufacturer's instructions) or a manual purification. The
 10 manual purification method was a modification of the standard alkaline lysis/cesium chloride preparation for plasmid DNA (see e.g., F.M. Ausubel et al., 1997, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY).

15 Briefly, for manual purification, cells were pelleted, and resuspended in GTE (50 mM glucose, 25 mM Tris-Cl (pH 8), and 10 mM EDTA) and lysozyme (50 mg/ml solution). This was followed by addition of NaOH/SDS (1% SDS and 0.2N NaOH) and then an ice-cold solution of 3M KOAc (pH 4.5-4.8). RnaseA was added to the filtered supernatant, followed by Proteinase K and 20% SDS. The DNA was precipitated with isopropanol,
 20 and then dried, and resuspended in TE (10 mM Tris, 1 mM EDTA (pH 8.0)). The BAC DNA was further purified by cesium chloride density-gradient centrifugation (Ausubel et al., 1997). Following isolation, the BAC DNA was hydrodynamically sheared using HPLC (Hengen et al., 1997, *Trends in Biochem. Sci.*, 22:273-274) to an insert size of 2000-3000 bp. After
 25 shearing, the DNA was concentrated and separated on a standard 1% agarose gel. A single fraction, corresponding to the approximate size, was excised from the gel and purified by electroelution (Sambrook et al., 1989).

30 The purified DNA fragments were then blunt-ended using T4 DNA polymerase. The blunt-ended DNA was then ligated to unique *Bst*XI-linker adapters (5' GTCTTCACCACGGGG (SEQ ID NO:) and 5' GTGGTGAAGAC (SEQ ID NO:) in 100-1000 fold molar excess. These

adapters were complimentary to the *Bst*XI-cut pMPX vector, whereas the *Bst*XI-cut vector was not self-complimentary. Therefore, the adapters would not concatemerize, and the cut vector would not ligate to itself. The linker-adapted inserts were separated from unincorporated linkers on a 1% agarose gel and purified using GeneClean (BIO 101, Inc., Vista, CA). The linker-adapted insert was then ligated to a modified pBlueScript vector to construct a "shotgun" subclone library. The vector contained an out-of-frame lacZ gene at the cloning site, which became in-frame in the event that an adapter-dimer was cloned. Such adapter-dimer clones gave rise to blue colonies, which were avoided.

Sequencing was performed using ABI377 automated DNA sequencing methods. Major modifications to the protocols are highlighted as follows. Briefly, the library was transformed into DH5-competent cells (GibcoBRL, DH5-transformation protocol). Transformed cells were plated onto antibiotic plates containing ampicillin and IPTG/X-gal. The plates were incubated overnight at 37°C. White colonies were identified, and plated to obtain individual clones for sequencing. Cultures were grown overnight at 37°C. DNA was purified using a silica bead DNA preparation method (Ng et al., 1996, *Nucl. Acids Res.*, 24:5045-5047). In this manner, 25 µg of DNA was obtained per clone.

Purified DNA samples were sequenced using ABI dye-terminator chemistry. The ABI dye terminator sequence reads were run on ABI377 machines, and the data were directly transferred to UNIX machines following lane tracking of the gels. All reads were assembled using PHRAP (P. Green, *Abstracts of DOE Human Genome Program Contractor-Grantee Workshop V*, Jan. 1996, p.157) with default parameters and quality scores. Each BAC was sequenced for ~3 X coverage. SEQ ID NOs for assembled contigs are shown in Table 3A, below.

TABLE 3A: BAC SEQUENCES

Genomic Sequence	SEQ ID NO: Range
RP11-666B20	719-765

RP11-702C13	766-808
RP11-723P10	809-869
RP11-831E18	870-899
RP11-899A17	900-927
RP11-932D22	928-978

Additional BAC sequences (GenBank (www.ncbi.nlm.nih.gov)) were also investigated as potentially containing gene or gene(s) involved in asthma and related diseases thereof.

5

TABLE 3B: BAC SEQUENCES

Genomic Sequence	SEQ ID NO:
AC003982	694
AC011216	695
AC023437	696
AC024021	697
AC024642	698
AC025641	699
AC025837	700
AC026331	701
AC026333	702
AC026336	703
AC026764	705
AC026869	704
AC048337	706
AC063926	707
AC069209	708
AC073527	709
AC073862	710
AC073912	711
AC073930	712
AC078925	713
AC078926	714
AC079031	715
AC079602	716
AC090147	717
AC090565	718
Z98941	979

EXAMPLE 6: GENE IDENTIFICATION

1. Gene Identification from clustered DNA fragments. DNA sequences corresponding to gene fragments in public databases (GenBank and human dbEST) and proprietary cDNA sequences (IMAGE consortium and direct selected cDNAs) were masked for repetitive sequences and

10

clustered using the PANGAEA Systems EST clustering tool (DoubleTwist, Oakland, CA). The clustered sequences were then subjected to computational analysis to identify regions bearing similarity to known genes. This protocol included the following steps:

- 5 a. The clustered sequences were compared to the publicly available UniGene database (NCBI) using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were: $E = 0.05$, $v = 50$, $B = 50$, where E was the expected probability score cutoff, V was the number of database entries returned in the reporting of the results, and B was the

10 number of sequence alignments returned in the reporting of the results (Altschul et al., 1990).

 b. The clustered sequences were compared to the GenBank database (NCBI) using BLASTN2 (Altschul et al., 1997). The parameters for this search were $E=0.05$, $V=50$, $B= 50$, where E , V , and B were defined as

15 above.

 c. The clustered sequences were translated into protein sequences for all six reading frames, and the protein sequences were compared to a non-redundant protein database compiled from GenPept, SWISSPROT, and PIR (NCBI). The parameters for this search were $E =$

20 0.05 , $V = 50$, $B = 50$, where E , V , and B were defined as above.

 d. The clustered sequences were compared to BAC sequences (see below) using BLASTN2 (Altschul et al., 1997). The parameters for this search were $E=0.05$, $V=50$, $B=50$, where E , V , and B were defined as above.

25 2. Gene Identification from BAC Genomic Sequence: Following assembly of the BAC sequences into contigs, the contigs were subjected to computational analyses to identify coding regions and regions bearing DNA sequence similarity to known genes. This protocol included the following steps:

30 a. Contigs were degapped. The contig sequences contained symbols that represented locations where the individual ABI sequence reads

had insertions or deletions (denoted by periods). Prior to automated computational analysis of the contigs, the periods were removed. The original contig sequences were held for future reference.

b. BAC vector sequences were masked within the sequence by using the program CROSSMATCH (P. Green, <http://chimera.biotech.washington.edu/UWGC>). Shotgun library construction (detailed above) left BAC vector sequences in the shotgun libraries. The CROSSMATCH program was used to compare the sequence of the BAC contigs to the BAC vector and to mask any vector sequence prior to subsequent steps. Masked sequences were marked by "Xs" in the sequence files, and were omitted during subsequent analyses.

c. *E. coli* sequences contaminating the BAC sequences were masked by comparing the BAC contigs to the entire *E. coli* genome.

d. Repetitive elements known to be common in the human genome were masked using CROSSMATCH (P. Green, University of Washington). In this implementation of CROSSMATCH, the BAC sequence was compared to a database of human repetitive elements (J. Jerka, Genetic Information Research Institute, Palo Alto, CA). The masked repeats were marked by "Xs" in the sequence files, and were omitted during subsequent analyses.

e. The location of exons within the sequence was predicted using the MZEF computer program (Zhang, 1997, *Proc. Natl. Acad. Sci.*, **94**:565-568) and GenScan gene prediction program (Burge and Karlin, *J. Mol. Biol.*, **268**:78-94).

f. The sequence was compared to the publicly available UniGene database (NCBI) using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were: E=0.05, V=50, B=50, where E was the expected probability score cutoff, V was the number of database entries returned in the reporting of the results, and B was the number of sequence alignments returned in the reporting of the results (Altschul et al., 1990).

g. The nucleotide sequence was translated into amino acid sequences for all six reading frames, and the amino acid sequences were compared to a non-redundant protein database compiled from GenPept, SWISSPROT, and PIR (NCBI). The parameters for this search were
5 E=0.05, V=50, B=50, where E, V, and B were defined as above.

h. The BAC DNA sequence was compared to a database of clustered sequences using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B were defined as above. The database of clustered sequences was
10 prepared utilizing a proprietary clustering technology (PANGAEA Systems, Inc.). The clustering program compiled cDNA clones derived from direct selection experiments (described below), human dbEST sequences mapping to the 12q23-ter region, proprietary cDNAs, GenBank genes, and IMAGE consortium cDNA clones.

i. The BAC sequence was compared to the BAC end sequences from the 12q23-ter region using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were E=0.05, V=50, B= 50, where E, V, and B were defined as above.
15

j. The BAC sequence was compared to the GenBank database (NCBI) using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B were defined as above.
20

k. The BAC sequence was compared to the STS division of GenBank database (NCBI) using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were E=0.05, V=50, B=50, where E, V, and B were defined as above.
25

l. The BAC sequence was compared to the Expressed Sequence Tag (EST) GenBank database (NCBI) using the BLASTN2 algorithm (Altschul et al., 1997). The parameters for this search were
30 E=0.05, V=50, B= 50, where E, V, and B were defined as above.

m. The exon prediction programs MZEF (Zhang, 1997, *Proc. Natl. Acad. Sci. USA* 94:565-568) and GenScan (Burge and Karlin, *J. Mol. Biol.*, 268:78-94) were also utilized to help identify the exons.

The results of BLAST searches of protein and nucleotide databases are summarized in Table 4. Column 1 lists the gene names, and column 2 lists the types of sequences (i.e., Gene, Express Sequence Tag (EST), etc.). Columns 3 and 4 list the SEQ ID NOs for the nucleotide and amino acid sequences, respectively. Column 5 lists the GenBank accession numbers. Column 6 lists the descriptions of the genes or ESTs relating to potential functions. Using this information, one of ordinary skill in the art is able to appreciate the roles of these genes and their relation to the disorders described herein. The seventh column lists the genetic markers, and the eighth column lists the corresponding BAC clones. The SEQ ID NOs corresponding to the BAC clones are shown in Tables 3A and 3B, above. It should be noted that 12q23-qter alternate splice variants are referred to herein using both short (e.g., 561.1, 561.2, etc.; see Table 4, column 1) and long (e.g., 561.nt1, 561.nt2; see Example 14) nomenclature.

TABLE 4: IDENTIFIED GENES

Gene Number	Gene Type	SEQ ID NO: (NT)	SEQ ID NO: (AA)	GenBank Accession #	Description	Marker	Genomic Seq
214.1	Gene	1	93	U14383	MUC8, Much 8	TH	RP11-702G13, AC079031
214.2	Gene	2	94	U14383	MUC8, Much 8	TH	RP11-702G13, AC079031
214.3	Gene	3	95	U14383	MUC8, Much 8	TH	RP11-702G13, AC079031
214.4	Gene	4	96	U14383	MUC8, Much 8	TH	RP11-702G13, AC079031
214.5	Gene	5	97	U14383	MUC8, Much 8	TH	RP11-702G13, AC079031
215.1	Gene	6	98	NM_004072	CMKLR1, Chemokine-like receptor 1	sts-U79526	
224.1	Gene	7	99	U17327	NOS1, Nitric oxide synthase 1, Neuronal	RK903904	
266.1	Gene	8	100	L07395	PPP1CC, Protein phosphatase 1, catalytic subunit, gamma isoform	SHGC11024	
283.1	Gene	9	101	AF055581	Lnk, Lymphocyte adaptor protein	SGC35065	
292.1	Gene	10	102	AF032437	MAPKAPK5, Mitogen activated protein kinase activated protein kinase gene	SGC34324	
298.1	Gene	11	103	D13540	PTPN11, Protein-tyrosine phosphatase 2C	WI-7628	
321.1	Gene	12	104	AB007447	Fin29, TRAF interacting Zn finger protein	A002Y44	
399.1	Gene	13	105	U14588	PXN Paxillin gamma	sts-AA002185	
399.2	Gene	14	106	U14588	PXN Paxillin gamma	sts-AA002185	
399.3	Gene	15	107	U14588	PXN Paxillin gamma	sts-AA002185	

567.1	EST	35		AA167552			siSG30525		
570.1	EST	36	123	H30072	Highly similar to Peptide transporter PTR2, [Saccharomyces cerevisiae]		SGC30248	AC023437, RP11-666B20	
570.2	EST	37	124	H30072	Highly similar to Peptide transporter PTR2, [Saccharomyces cerevisiae]		SGC30248	AC023437, RP11-666B20	
571.1	EST	38		N20163			sis-N20163		
572.1	EST	39		AF052172			Cda0a101		
575.1	EST	40	125	R24284			SGC34088		
577.1	Gene	41	126	J05158	CPN, Carboxypeptidase N		siSG16387		
579.1	EST	42		H20731			WI-15018		
581.1	Gene	43		H23544	RAN, TC4, Ras-like protein		WI-16177	AC073912, RP11-899A17	
581.2	Gene	44		H23544	RAN, TC4, Ras-like protein		WI-16177	AC073912, RP11-899A17	
583.1	EST	45	127	A006016			A006016		
584.1	EST	46		H64839			H64839		
586.1	EST	47		AA180186			siSG30906		
587.1	EST	48		T50225			siSG26056		
589.1	Gene	49	128	AA025934	CAGH32		siSG1702		
590.1	EST	50		N59820			sis-N59820		
592.1	Gene	51	129	AF045458.1	JLK1, Homo sapiens serine/threonine kinase		IB2452		
594.1	EST	52	130	AA252357			AA252357		
595.1	Gene	53	131	AF116238.1	PUS1, pseudouridine synthase 1		siSG4720		
596.1	EST	54	132	AA001424			sis-AA001424		
601.1	EST	55		R50113			R50113		
603.1	EST	56		H94865			sis-H94865		
604.1	EST	57		A006R19			A006R19		
605.1	EST	58	133	N23648	similar to ZN91_HUMAN ZINC FINGER PROTEIN 91		SGC34278		

505.2	EST	59		N23648	similar to ZN91_HUMAN ZINC FINGER PROTEIN 92	SGC34278	
506.1	EST	60	134	A004B47	Similar to DNA Polymerase epsilon, catalytic subunit	A004B47	
508.1	EST	61	135	AB014592		siSG40199	
511.1	EST	62	136	A005Q05		A005Q05	
515.1	Gene	63	137	D63997	GCP170, Golgi membrane protein	CDA1JF08	
517.1	Gene	64	138	U09368	ZNF140	siSG31494	
518.1	EST	65	139	R44594	ZNF84	siSG40222	
521.1	EST	66	140	R81342	ZNF10	sis-R81342	
522.1	EST	67		H65839		sis-H65839	
590.1	EST	68	141	AA812723		siSG60065	
592.1	EST	69		R24284	similar to reverse transcriptase homolog [<i>H. sapiens</i>]	WI-AF856	
593.1	EST	70	142	AA678190		siSG52343	
594.1	EST	71		AA897697		siSG54325	
595.1	EST	72		AA705809		siSG63473	
597.1	EST	73		AA889526		siSG53307	
598.1	GENE	74	143	AF104260.1	hhlw	siSG53541	AC025837, RP11-831E18, AC090147, AC090565
599.1	GENE	75	144	N49217	SFRS8, Splicing factor, arginine/serine-rich 8, (suppressor-of-white-apricot Drosophila homolog)	siSG43910	
702.1	GENE	76	145	X89576	MMP17, matrix metalloproteinase 17 (membrane-inserted)	sisX89576	RP11-932D22, RP11-723P10
705.1	EST	77	146	AA846540		siSG54842	
707.1	EST	78	147	AA223499		siSG48386	
707.2	EST	79	148	AA223499		siSG48386	

EXAMPLE 7 : cDNA CLONING

1. Construction and screening of cDNA libraries: Directionally-cloned cDNA libraries from normal lung and bronchial epithelium were constructed using standard methods (Soares et al., 1994, *Automated DNA Sequencing and Analysis*, Adams et al. (eds), Academic Press, NY, pp. 110-114). Total and cytoplasmic RNAs were extracted from tissue or cells by homogenizing the sample in the presence of guanidinium thiocyanate-phenol-chloroform extraction buffer (e.g., Chomczynski and Sacchi, 1987, *Anal. Biochem.* **162**:156-159) using a polytron homogenizer (Brinkman Instruments, <http://www.brinkmann.com>). Poly (A)+ RNA was isolated from total/cytoplasmic RNA using dynabeads-dT according to the manufacturer's recommendations (Dynal, Inc., <http://www.dynal.com>). The double stranded cDNA was then ligated into the plasmid vector pBluescript II KS+ (Stratagene, <http://www.stratagene.com>), and the ligation mixture was transformed into *E. coli* host DH10B or DH12S by electroporation (Soares, 1994). Following overnight growth at 37°C, DNA was recovered from the *E. coli* colonies after scraping the plates as directed for the Mega-prep kit (QIAGEN). The quality of the cDNA libraries was estimated by counting a portion of the total number of primary transformants, determining the average insert size, and calculating the percentage of plasmids without cDNA insert. Additional cDNA libraries (human total brain, heart, kidney, leukocyte, and fetal brain) were purchased from Life Technologies (Bethesda, MD).
- 25 cDNA libraries, both oligo (dT) and random hexamer-primed, were used to isolate cDNA clones mapped within the disorder critical region. Four 10 x 10 arrays of each of the cDNA libraries were prepared as follows. The cDNA libraries were titered to 2.5×10^6 using primary transformants. The appropriate volume of frozen stock was used to inoculate 2 L of LB/ampicillin (100 µg/µl). Four hundred aliquots containing 4 ml of the inoculated liquid culture were generated. Each tube contained about 5000 cfu (colony forming units). The tubes were incubated at 30°C overnight with

shaking until an OD of 0.7-0.9 was obtained. Frozen stocks were prepared for each of the cultures by aliquotting 300 μ l of culture and 100 μ l of 80% glycerol. Stocks were frozen in a dry ice/ethanol bath and stored at -70°C. DNA was isolated from the remaining culture using the QIAGEN spin mini-prep kit according to the manufacturer's instructions. The DNAs from the 400 cultures were pooled to make 80 column and row pools. Markers were designed to amplify putative exons from candidate genes. Once a standard PCR condition was identified and specific cDNA libraries were determined to contain cDNA clones of interest, the markers were used to screen the arrayed library. Positive addresses indicating the presence of cDNA clones were confirmed by a second PCR using the same markers.

Once a cDNA library was identified as likely to contain cDNA clones corresponding to a transcript of interest from the disorder critical region, it was used to isolate a clone or clones containing cDNA inserts. This was accomplished by a modification of the standard "colony screening" method (Sambrook et al., 1989). Specifically, twenty 150 mm LB plus ampicillin agar plates were spread with 20,000 cfu of cDNA library. Colonies were allowed to grow overnight at 37°C. Colonies were then transferred to nylon filters (Hybond from Amersham-Pharmacia, or equivalent) and duplicates prepared by pressing two filters together essentially as described (Sambrook et al., 1989). The "master" plate was then incubated an additional 6-8 hr to allow the colonies additional time to grow. The DNA from the bacterial colonies was then bound to the nylon filters by incubating the filters with denaturing solution (0.5 N NaOH, 1.5 M NaCl) for 2 min, and neutralization solution (0.5 M Tris-Cl pH 8.0, 1.5 M NaCl) for 2 min (twice). The bacterial colonies were removed from the filters by washing in a solution of 2 X SSC/2% SDS for 1 min while rubbing with tissue paper. The filters were air-dried and baked under vacuum at 80°C for 1-2 hr to crosslink the DNA to the filters.

cDNA hybridization probes were prepared by random hexamer labeling (Fineberg and Vogelstein, 1983, *Anal. Biochem.* 132:6-13). For

small fragments, probes were prepared using gene-specific primers and omitting random hexamers in the reaction. The colony membranes were pre-washed in 10 mM Tris-Cl pH 8.0, 1 M NaCl, 1 mM EDTA, and 0.1% SDS for 30 min at 55°C. Following the pre-wash, the filters were pre-hybridized
 5 in more than 2 ml/filter of 6 X SSC, 50% deionized formamide, 2% SDS, 5 X Denhardt's solution, and 100 mg/ml denatured salmon sperm DNA, at 42°C for 30 min. The filters were then transferred to hybridization solution (6 X SSC, 2% SDS, 5 X Denhardt's, and 100 mg/ml denatured salmon sperm DNA) containing denatured α -³²P-dCTP-labeled cDNA probe, and incubated
 10 overnight at 42°C.

The following morning, the filters were washed under constant agitation in 2 X SSC/2% SDS at RT (room temperature) for 20 min, followed by two washes at 65°C for 15 min each. A final wash was performed in 0.5 X SSC/0.5% SDS for 15 min at 65°C. Filters were then wrapped in plastic
 15 wrap and exposed to radiographic film. Individual colonies on plates were aligned with the autoradiograph, and positive clones were inoculated into a 1 ml solution of LB Broth containing ampicillin. After shaking at 37°C for 1-2 hr, aliquots of the solution were plated on 150 mm plates for secondary screening. Secondary screening was identical to primary screening (above),
 20 except that it was performed on plates containing ~250 colonies, so that individual colonies could be clearly identified. Positive cDNA clones were characterized by restriction endonuclease cleavage, PCR, and direct sequencing to confirm the sequence identity between the original probe and the isolated clone.

25 4. Gene Identification in region 12q23-qter by Direct cDNA Selection: Direct cDNA selection is a powerful technique for the identification of genes mapping to a particular genomic interval. It involves hybridizing genomic DNA (in this case, BACs) from a region of interest to pools of cDNAs derived from various tissue sources. The procedure permits
 30 the rapid isolation of cDNAs, and obviates the need for extensive screening of cDNA libraries. The tissues used in this study included unstimulated Th2

cells, Th2 cells stimulated with TPA, bronchial smooth muscle cells, unstimulated Th0 cells, Th0 stimulated with anti CD3 and TPA, pulmonary artery endothelium cells, lung microvascular endothelial cells, bronchial epithelium cells, normal and asthmatic lung, small airway epithelium cells, pulmonary artery smooth muscle cells, and lung fibroblasts. These cell types have been implicated in the pathophysiology of asthma and were expected to express genes involved in the asthmatic inflammatory response. In addition, RNA isolated from brain cells was used, because brain cells expresses a diverse array of genes.

Cytoplasmic RNA was isolated as described by Sambrook et al, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY. Approximately 400-600 µg of cytoplasmic RNA was isolated from 50 million cells. Total RNA was isolated from normal and asthmatic lung tissue using TRIzol Reagents (GibcoBRL), which are ready-to-use monophasic solutions of guanadinium isothiocyanate and phenol (P. Chomczynski and N. Sacchi, 1987, *Anal. Biochem.* **162**:156-159; P. Chomczynski et al., 1987, *J. NIH Res.* **6**:83; D. Simms et al., 1993, *Focus* **15**:99; P. Chomczynski, 1993, *BioTechniques* **15**:532). Five hundred milligrams of frozen tissue was crushed into a fine powder using a Bessman tissue pulverizer (Fisher Scientific). The TRIzol Reagents were mixed with the crushed tissue according to the manufacturer's recommendations.

To ascertain whether there was genomic DNA or heteronuclear RNA contamination, PCR and RT/PCR were performed. PCR analysis was performed using primers (Research Genetics) that amplified STS markers from chromosomes 2 (D2S2358), 7 (D7S2776 and D7S685), 10 (D10S228 and D10S1755), and 20 (D20S905 and D20S95). All PCR reactions were performed in a final volume of 25 µl, containing 1 µl of RNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 200 mM each dNTPs, 10 µM of each primer, and 1 U Taq DNA polymerase (Perkin Elmer). A Perkin Elmer 9600 cycler was used for amplification as follows: 30 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C for 30 cycles. RT/PCR analysis

was performed using the SuperScript One-Step RT-PCR System (Gibco-BRL, Rockville, MD) according to the manufacturer's recommendations. All PCR and RT/PCR products were evaluated by electrophoresis on a 1% agarose gel.

5 Poly (A)+ RNA was prepared from the total RNA isolated from the human primary cells and lung tissues using Dynabeads Oligo(dT) according to the manufacturer's recommendations (DynaI, Lake Success, NY). Approximately 4 µg of messenger RNA was isolated from 150 µg of total RNA for each cell type and tissue source. Total RNA isolated from brain
10 tissue was purchased from CLONTECH (Palo Alto, California), and poly(A)+ RNA was prepared from this material using Dynabeads Oligo(dT), described above. Oligo dT and random primed cDNA pools were generated from the mRNA isolated from each cell type and tissue source. Briefly, 2.0 µg mRNA was mixed with oligo(dT) primer in one reaction. In another reaction, 2.0 µg
15 mRNA was mixed with random hexamers, and converted to double stranded complementary DNA using the SuperScript Choice System for cDNA Synthesis (Gibco-BRL, Rockville, MD) according to the manufacturer's recommendations.

Four different paired phosphorylated cDNA linkers (Table 5) were
20 annealed by mixing a 1:1 ratio of the paired linkers (10 µg each), incubating the mixture at 65°C for 5 min, and allowing the mixture to cool to RT for 30 min. The annealed linkers were ligated to the oligo(dT) and random-primed cDNA pools from various tissue and cell sources (Table 5) according to manufacturer's instructions (GibcoBRL). The linker sequence provided a tag
25 to identify the RNA from the particular cell types.

TABLE 5: PAIRED LINKERS

<u>Paired linkers</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Cell/Tissue Type</u>
OLIGO 3	5'CTC GAG AAT TCT GGA TCC TC3'		Th2/unstimulated (dT+rp)
OLIGO 4	5'TTG AGG ATC CAG AAT TCT CGA G3'		Th0/stimulated/anti CD3 (dT+rp)
			Pulmonary artery endothelium cells (dT+rp)
			Lung microvascular
			Endothelial cells (dT+rp)
			Bronchial epithelium cells (dT+rp)
OLIGO 5	5'TGT ATG CGA ATT CGC TGC GCG3'		Normal Lung (dT+rp)
OLIGO 6	5'TTC GCG CAG CGA ATT CGC ATA CA3'		Athmatic lung (dT+rp)
			Th2/stimulated/TPA (dT+rp)
			Bronchial smooth muscle cells (dT+rp)
OLIGO 9	5'CCT ACG GAA TTC TCA CTC AGC3'		Brain (dT+rp)
OLIGO 10	5'TTG CTG AGT GAG AAT TCC GTA GG3'		Th0/unstimulated (dT+rp)
			Pulmonary artery smooth muscle cells (dT+rp)
OLIGO 11	5'GAA TCC GAA TTC CTG GTC AGC3'		Lung fibroblasts (dT+rp)
OLIGO 12	5'TTG CTG ACC AGG AAT TCG GAT TC3'		Th0/stimulated/ TPA (dT+rp)
			Small airway epithelium cells (dT+rp)

The cDNA pools were evaluated for length distribution by PCR amplification using 1 µl of a 1:1, 1:10, and 1:100 dilution of the ligation reaction. All PCR reactions were performed in a final volume of 25 µl, containing 1 µl of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 200 mM of each dNTP, 10 µM of each primer, and 1 U Taq DNA polymerase (Perkin Elmer). A Perkin Elmer 9600 cycler was used to for amplification as follows: 30 seconds at 94°C, 30 seconds at 55°C, and 2 minutes at 72°C for 30 cycles. The length distribution of the amplified cDNA pools was evaluated by electrophoresis on a 1% agarose gel. The PCR reaction that gave the best representation of the random primed and oligo dT primed cDNA pools was scaled-up to yield ~2-3 µg of each cDNA pool. This represented a 1 X PCR reaction for the starting cDNA pools.

Twenty BACs (Table 6) that spanned the 15 cM critical region between markers D12S1609 and D12S357 were pooled in equimolar amounts. One microgram of the isolated genomic DNA was labeled with biotin 16-UTP by nick translation in accordance with the manufacturer's instructions (Boehringer-Mannheim). The incorporation of biotin was monitored by standard methods (Del Mastro and Lovett, 1996, *Methods in Molecular Biology*, Humana Press Inc., NJ).

TABLE 6: BACs SPANNING THE 15 cM REGION

0753B07
0666B20
0687F10
0820N16
0899A17
0716I10
0839D11
0894M06
0696L08
0979G13
0723P10
0932D22
0825K21

0866B05
0750I23
0831E18
0761L21
0702C13
0739N03
1064I09

Direct cDNA selection was performed using standard methods (Del Mastro and Lovett, 1996, Methods in Molecular Biology, Humana Press Inc., NJ). Briefly, 1 µg of each cDNA pool was placed into individual PCR tubes.

5 A total of 30 direct selection experiments were arrayed onto a PCR plate. Suppression of high copy repeats, ribosomal RNA, and plasmid DNA in the cDNA pools was performed to a Cot_{20} . One hundred nanograms of biotinylated BAC DNA was mixed with the suppressed cDNAs, and hybridized in solution to a Cot_{200} . The biotinylated DNA and the cognate
10 cDNAs were then captured on streptavidin-coated paramagnetic beads. The beads were washed and the primary selected cDNAs were eluted. The products from the first round of direct selection were PCR amplified using appropriate primers (shown in Table 5), and a second round of direct selection was performed.

15 GTP-Binding Nuclear Protein RAN (TC4, a gene that maps within the 7.6 cM critical region) was used to monitor the enrichment during the two rounds of direct selection. The enrichment of the TC4 was monitored in the starting, primary, and secondary selected material of the fifteen oligo dT and random primed cDNA pools. The random primed product of the second
20 round of direct selection (the secondary selected material) from lung microvascular endothelial cells, Th0/unstimulated cells, lung fibroblast cells, Th2/unstimulated cells, pulmonary artery endothelium cells, normal lung, small airway epithelium cells, bronchial epithelium cells, Th0 cells stimulated with TPA, and oligo dT primed Th0 cells stimulated with TPA was PCR-
25 amplified with modified primers (Table 7, below). These primers were used for two rounds of direct cDNA selection.

TABLE 7: MODIFIED OLIGONUCLEOTIDES

	Modified Oligonucleotides	SEQ ID NO	Sequence
5	OLIGO 3		5' CUA CUA CUA CUA CTC GAG AAT TCT GGA TCC TC 3'
	OLIGO 5		5' CUA CUA CUACUATGT ATG CGA ATT CGC TGC GCG 3'
	OLIGO 9		5' CUA CUA CUA CUA CCT ACG GAA TTC TCA CTC AGC 3'
	OLIGO 11		5' CUA CUA CUA CUA GAA TCC GAA TTC CTG GTC AGC 3'

The amplified material was cloned into the UDG vector pAMP10 (GibcoBRL) in accordance with the manufacturer's recommendations. Four hundred and eighty clones were picked from each transformed source and arrayed into five 96-well microtiter plate. Each selected cDNA library was stamped, in duplicate, in high density format onto Hybond N+ nylon membrane (Amersham). The bacteria were grown overnight at 37°C, and the membranes were processed as recommended by the manufacturer.

To identify which of the clones represented common contaminants (e.g., high copy repeats and ribosomal RNA), a radiolabeled probe containing 1 µg of Cot₁ DNA and 0.5 µg ribosomal DNA was hybridized at 65°C to the high density filters (Sambrook et al, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY). The filters were washed three times in buffer (0.1 X SSC/0.1% SDS) at 65°C, and were autoradiographed. Those cDNAs that showed duplicate signals were scored as background contaminants. The remaining clones were re-arrayed into 96-well microtiter plates. A total of twenty-three 96-well microtiter plates containing 2208 secondary selected clones were sequenced. This included three 96-well microtiter plates from all the random primed selections. Except, only two plates were included for the Th0 cells stimulated with TPA, and only one plate was included for the Th0 cells stimulated with TPA from the oligo dT selection. All cDNA clones were sequenced using M13 dye primer terminator cycle sequencing kits (Applied Biosystems). Data was collected by the ABI 377 automated fluorescence sequencer (Applied Biosystems).

Clones representing other contaminants, such as high copy repeats, ribosomal RNA, plasmid DNA, mitochondrial DNA, and *E. coli* and yeast DNA that were not identified in the hybridization process were removed from the dataset using *in silico* methods. This produced a set of cDNA clones
5 corresponding to SEQ ID NO:980 to SEQ ID NO:1766, disclosed herein. These clones were clustered using PANGEA System's EST Clustering Tool (Oakland, CA), and analyzed with BLASTN, BLASTX, and FASTA programs. This allowed the assembly of full-length gene sequences. The
10 direct selected clones were combined with the ESTs homologous to BAC sequences, BAC end sequences, and sequence within the public domain (dbEST and GenBank), and then clustered using the PANGEA Systems EST Clustering Tool. The clustered sequences (i.e., consensus sequences) correspond to SEQ ID NO:1767 to SEQ ID NO:4687, disclosed herein. *In silico* and hybridization techniques were used to map the direct selected
15 cDNAs to the 15 cM region. Using well-established sequencing techniques, one skilled in the art could extend these candidate clones to map back the region into a full-length gene.

EXAMPLE 8: EXPRESSION ANALYSIS

In order to characterize the expression of genes mapping to the
20 12q23-qter region, a series of experiments were performed. First, oligonucleotide primers were designed for PCR and RT-PCR reactions to amplify cDNA, EST, or genomic DNA could be amplified from a pool of DNA molecules or RNA population. The PCR primers were used in a reaction containing genomic DNA to verify that they generated a product of the
25 predicted size, based on the genomic sequence. The length, in nucleotides, of the processed transcript or messenger RNA (mRNA) was determined by Northern analysis (Sambrook et al, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor NY). Probes were generated using one of the methods described below.

30 Briefly, sequence verified IMAGE consortium cDNA clones were digested with appropriate restriction endonucleases to release the insert.

The restriction digest was electrophoresed on an agarose gel and the bands containing the insert were excised. The gel piece containing the DNA insert was placed in a Spin-X (Corning Costar Corporation, Cambridge, MA) or Supelco spin column (Supelco Park, PA) and spun at high speed for 15 min.

- 5 The DNA was ethanol precipitated and resuspended in TE. Alternatively, PCR products obtained from genomic DNA or RT-PCR were purified as described above. Inserts purified from IMAGE clones were random primer labeled (Feinberg and Vogelstein) to generate probes for hybridization. Probes from purified PCR products were generated by incorporation of α -³²P-dCTP in second round of PCR. Commercially available Multiple Tissue Northern blots (CLONTECH, Palo Alto, CA) were hybridized and washed under conditions recommended by the manufacturer.
- 10

Figures 6A-6U show Northern blots illustrating the expression of the indicated genes in various tissues. With the exception of Gene 214 (Figure 6A), all blots were Multiple Tissue Northern Blots (CLONTECH, Palo Alto, CA). The tissues included: 1) brain; 2) heart; 3) skeletal muscle; 4) colon; 5) thymus; 6) spleen; 7) kidney; 8) liver; 9) small intestine; 10) placenta; 11) lung; and 12) peripheral blood leukocytes. Size standards (Kb) are indicated to the left of each blot. Figure 6A shows the Northern blot for Gene 214, which includes poly (A)+ selected RNA from 1) a lymphoblast cell line from an asthmatic individual; 2) lung; and 3) trachea.

RT-PCR was used as an alternate method to Northern blotting to detect mRNAs with low levels of expression. Total RNA from multiple human tissues was purchased from CLONTECH (Palo Alto, CA), and genomic DNA was removed by DNaseI digestion. The Superscript[®] Preamplification System for First strand cDNA synthesis (Life Technologies, Gaithersburg, MD) was used according to manufacturer's directions, with oligo(dT) or random hexamers to synthesize cDNA from the DNaseI treated total RNA. Gene specific primers were used to amplify the target cDNAs in a 30 μ l PCR reaction containing 0.5 μ l of first strand cDNA, 1 μ l sense primer (10 μ M), 1 μ l antisense primer (10 μ M), 3 μ l dNTPs (2 mM), 1.2 μ l

MgCl₂ (25 mM), 3 µl 10X PCR buffer, and 1 U Taq Polymerase (Perkin Elmer). The PCR reaction included a denaturation step at 94°C for 4 min, followed by 30 cycles at 94°C for 30 sec, 58°C for 1 min, and 72°C for 1 min, and an extension step at 72°C for 7 min. PCR products were analyzed on
5 agarose gels.

The 12q23-qter genes are shown in Table 4; the nucleotide sequences correspond to SEQ ID NO:1 to SEQ ID NO:92, the encoded amino acid sequences correspond to SEQ ID NO:93-155, and the BAC nucleotide sequences correspond to SEQ ID NO:694 to SEQ ID NO:979, as
10 disclosed herein.

EXAMPLE 9: MUTATION ANALYSIS

In order to conduct mutation analysis, the genomic structure of Gene 214, Gene 224, Gene 422, Gene 436, Gene 449, Gene 454, Gene 515, Gene 561, Gene 570, Gene 581, Gene 698, Gene 702, Gene 722, Gene
15 748, Gene 751, Gene 757 and Gene 848 was determined. For genes with previously unidentified exon-intron boundaries, the cDNA sequences were compared to genomic sequence from the BACs. The precise intron-exon junctions were determined based on the consensus sequences at splice junctions. The exon prediction programs MZEF (Zhang, 1997, *Proc. Natl. Acad. Sci.*, 94:565-568) and GenScan (Burge and Karlin, 1997, *J. Mol. Biol.*,
20 268:78-94) were also utilized to identify the exons.

Disorder associated candidate genes (Table 4) were identified using the above procedures, and exons from these genes were subjected to mutation detection analysis. A combination of fluorescent single stranded
25 confirmation (SSCP) analysis (ABI), DNA sequencing, and other sequence analysis methods described herein were utilized to precisely identify and determine nucleotide sequence variants. SSCP analysis was used to screen individual DNA sequences for variants. Briefly, PCR was used to generate templates from unrelated asthmatic individuals that showed
30 increased sharing for the 12q23-qter chromosomal region, and contributed towards linkage. Non-asthmatic individuals were used as controls.

Enzymatic amplification of genes within the asthma region of 12q23-qter was accomplished using primers flanking each exon and the putative 5' regulatory elements of each gene. The primers were designed to amplify each exon, as well as 15 or more base pairs of each intron on either side of the splice site. The forward and the reverse primers had two different dye colors to allow analysis of each strand, and independent confirmation of variants. PCR reactions were optimized for each exon primer pair. Buffer and cycling conditions were specific to each primer set. PCR products were denatured using a formamide dye, and electrophoresed on non-denaturing acrylamide gels with varying concentrations of glycerol (at least two different glycerol concentrations).

Primers utilized in fluorescent SSCP experiments to screen coding and non-coding regions of Gene 214, Gene 224, Gene 422, Gene 436, Gene 449, Gene 454, Gene 515, Gene 561, Gene 570, Gene 581, Gene 698, Gene 702, Gene 722, Gene 748, Gene 751, Gene 757 and Gene 848 for polymorphisms are provided in Table 8. Column 1 lists the genes targeted for mutation analysis. Column 2 lists the specific exons analyzed. Column 3 lists the assigned primer names. Columns 4 and 5 list the forward primer sequences and the reverse primer sequences, respectively. The genes listed in column 1 of Table 8 correspond to the gene identifiers in column 1 of Table 4.

TABLE 8: SSCP PRIMERS

Primers used in SSCP experiments						
		SSCP Assay	SEQ ID NO:	Forward Sequence	SEQ ID NO:	Reverse Sequence
Gene	Exon					
454	A	55_454_A_F 56_454_A_R		TGGCCCTGTG CAGGAAGTA		CTGCAGAGATCTGGGTCTC
454	B	57_454_B_F 58_454_B_R		TTGATGCTTTCCCATGTCTG		GGAGAATGCTACGAGGTGCT
454	C	59_454_C_F 60_454_C_R		TCAAAGGCCTTGCAATTTCT		GTCCGCATTTCTGCTTCTTC
454	D	61_454_D_F 62_454_D_R		TCCCACTCTGTCACTCTTC		GAGGCTGAAGACCTGACCTG
454	E	63_454_E_F 64_454_E_R		CCTCTCCGCAGTTCTTTTAC		GAGGGCCACTGTGTCTGTCT
454	F	65_454_F_F 66_454_F_R		GTATCCCAAGAGCAAGCCA		AACTAAGACAGCCAGGCAAGC
454	G	67_454_G_F 68_454_G_R		ATGGAACCTCTCCACCACAC		TCCAGTGTACAAAGCACCA
454	H	69_454_H_F 70_454_H_R		CTGGCTATGCAGGGAGATGT		GTGAGTTTGACCTGGGCT
454	K	71_454_K_F 72_454_K_R		CCAGAACCAGCACATTTCA		AGGCTGAGACCAAAACCGCT
454	L	73_454_L_F 74_454_L_R		AACCAACAATTGCACGTTGA		TGTCGATGAGGAAGTCCGATG
454	M	75_454_M_F 76_454_M_R		CAGCGCTTGTCTGCATTCT		GGAATCTCTCCGTGTCTTGG
454	N	77_454_N_F 78_454_N_R		TGATAATTCTGTACAAAATGGGTAA		CTTTGTAAATCCATCAGTTTTC
454	O1	79_454_O_F 80_454_O_R		CCTAGAACCCTGAGGCTTGTG		CTGTGGCTCTCAGGGAGTTG
454	O2	81_454_O_F 82_454_O_R		GGTGCCAGTGTGGAAGATG		AGGTGGCGTAGCACCTGTAG
454	O3	83_454_O_F 84_454_O_R		CACCACCTCAGAGCTGTTC		ACTGCCCTTCACTCTTTGGA
454	O4	85_454_O_F 86_454_O_R		CCAGGACATGGCTGACTTTG		ACAGACAGGATTTCCGCTTG
454	AA	1959_454_AA_F 1960_454_AA_R		GAATATTCCAATTTTGCCTGG		CCGAGGAAAGTGGAGTTGAG
454	AA	1961_454_AA_F 1962_454_AA_R		CCTGTTTTCCTTTGAGTCCA		TACTCTCCACCCTCTCTGCG
454	AA	1963_454_AA_F 1964_454_AA_R		CCTGGTGATCTTTGGCTGAT		ACAACCTTTTATTCAGCCCC
454	AA	1965_454_AA_F 1966_454_AA_R		GGGGAGATCTTCATTACCCA		GTGTTGAGAGGATGGGCATT
454	AA	1967_454_AA_F 1968_454_AA_R		GGGAAAAGGGAGAAATTTCTAAA		CCCTCCAGTAAGTGCACAAA
454	AA	1969_454_AA_F 1970_454_AA_R		GCAGTCATTGGAGAGCTTG		GGAAAAGATGATCAGCTGGAA
757	A	1750_757_A_F 1751_757_A_R		GAGCAGGGGTGGAGAGCC		CAGGTTGGGCATACGAGTCA
757	A	1752_757_A_F 1753_757_A_R		GCAAGGACATCGGCTACAA		ATAATCGGGGAGCATTGAG
757	A	1778_757_A_F 1779_757_A_R		TGCACCGAGCAGGTCTCTAC		GTCTTTCAGCGGGTGTCTC
757	A	1780_757_A_F 1781_757_A_R		AACTACCTGTGCATGGAGGC		GAAGGTGAGCACCGGTGAAG

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757	A	1758 757 A F 1759 757 A R	CGTGTCACCTTCCTCATC	GTGAGGACCACCCACCAC
757	A	1760 757 A F 1761 757 A R	CTGTGGTGGTGGTCCTC	GTAGCAGGCCAGGGGAAT
757	A	1782 757 A F 1783 757 A R	TCTGCTACGTGGGCAGCAT	CCATGTTGAGGCGTTCGTAA
757	A	1784 757 A F 1785 757 A R	CTCTGTGCTGTACACCGTGC	GGTTTCTCCGGCTCTTCTT
757	A	1786 757 A F 1787 757 A R	CCTCAAGACTCTGCAGTCC	CACAACCAAGAAAAGCACCA
757	A	1788 757 A F 1789 757 A R	AAATATGAGATCCCTGCCCA	CTTCGCTGGAAAACCAAAAC
757	A	1768 757 A F 1769 757 A R	TGAAATTCAGGATGCTGTGA	TTGCAAGCAGTTATCTGTCC
757	A	1770 757 A F 1771 757 A R	TTGAGTTGGCTTTGCTACCC	TGTGAGGTTTGTGGAGGTTT
757	A	1772 757 A F 1773 757 A R	CTGCAAGACAGAAAACCTCCA	TCCACAAATCAGTCCAAACG
757	A	1774 757 A F 1775 757 A R	TAATGGAACCAAGCCCAATG	CAATATACACACGACAGAAACC
757	A	1776 757 A F 1777 757 A R	TGCCAGGAAAGAGTGGTTTC	GCTAGAAGCACAAACCCACGA
561	A	1530 561 A F 1531 561 A R	AGGGTATAGGATGCACGCC	CTCCACCACACACGGGAT
561	B	937 561 B F 938 561 B R	ACACACATTTCCACCACCAAA	CATGAACGTGGGAAAGGCT
561	B	939 561 B F 940 561 B R	CCGGACTCAAAGTGAGCAGT	ATTTCACCTGTGCACACCCCT
561	C	941 561 C F 942 561 C R	CATGACCAACGTGCTTTGAC	ATCTGGCTACCGGATCT
561	C	943 561 C F 944 561 C R	GTCAGGAGAGCGCTATTGGA	AACAGGACAAACTGGCCAAAC
561	D	945 561 D F 946 561 D R	CCTCCAGCTTCAATAACCCA	AAATCCACCTTCTCCTCGT
561	E	947 561 E F 948 561 E R	TGTGCTCTCCAGAGCCCTCTAA	GGAGCCCTGCCTATCTATC
561	F	949 561 F F 950 561 F R	CTGTGTTGGCTGGGTGATAA	GGCACTGTTGTCGGTGATG
561	F	951 561 F F 952 561 F R	GAGAGCACATCCTGGACCTC	TTCATGCGTGTCTCCTTGTC
561	F	953 561 F F 954 561 F R	GCCACCAGGATGGGGAAC	TCTGCGTGATGTTGTCCAC
561	F	955 561 F F 956 561 F R	GTGGGCAAGGACGTGGTG	CTCCCTTTGCTCCAGCGG
561	F	957 561 F F 958 561 F R	CACGTATCTTCTCTCAACGA	GGAAGGACACAGGGCTCAC
561	G	1532 561 G F 1533 561 G R	ACCGAATGATCTCGTTTCCA	AAAACCTACCCCTCTGCCCCT
561	G	1534 561 G F 1535 561 G R	CACCCCAACAAGATGTTACC	AGTGATCAGGGCTGGAAGAG
561	H	961 561 H F 962 561 H R	GGCTCCCAATTGCAGGAC	TGATTGGGGTGCAGGTCCTC
561	H	963 561 H F 964 561 H R	ACTCTGAGTGTCTGCCGT	CTGTGGCTGTGGCAGGAT
561	H	1536 561 H F 1537 561 H R	CACGCCAGGATGGATGAG	GACTGAGGAGGCCACCGAG
561	I	967 561 I F 968 561 I R	GTAGCTGAAGTGGCCCTG	CCACCAGGAGGATGGTGT
561	J	969 561 J F 970 561 J R	TGTAGGATCGGGAGGAG	AGCTACTCTGGGACGGAG
561	K	971 561 K F 972 561 K R	ATGCTGGGAGACTTACGAC	TTTGCTTAGCGGAAAATGCT
561	L	973 561 L F 974 561 L R	CACGCTCCTCAGTTAGGCTC	CACCTTGATGATCTGGCCTT
561	L	975 561 L F 976 561 L R	AGACCGCCTTCTCCAGACT	GTGATACCCCTGTTGCCAGT

422	C	1859 422 C F 1860 422 C R	TTATCTGGGCAGGGTGTGT		CCCATTCAGAGGAGTGAGA
422	D	1861 422 D F 1862 422 D R	CTGGCAGACCGATTGAAC		GGCAGGCACCTCAATTTTC
422	E	1863 422 E F 1864 422 E R	GTGAGGGCTGACCTATTGCT		CGGCCTACTGAGAACCAACT
422	F	1865 422 F F 1866 422 F R	TTCTTCTTGCCCCAGATTGT		TGAGATGAGGCAGATAGAGGTG
422	F	1867 422 F F 1868 422 F R	AAGGCACACAAGAACCTGGA		AGGTGGCATCACCTGCACCTC
436	A	1549 436 A F 1550 436 A R	CCTAGAGGGTCATOGTTCCC		TGCTACTCGAACAGGAAGGC
436	A	1551 436 A F 1552 436 A R	ACCCAGACCGACTAGGGGAC		GACCGAGGCCACGGATGAG
436	B	1553 436 B F 1554 436 B R	TTCCCCATCAATTCAATACC		TCAGGCCACGTCAATCATTA
436	C	1555 436 C F 1556 436 C R	TTTCTTGGCTCTCCGTGAGT		GAGCGAAAAGAAAGTCCACG
436	D	1557 436 D F 1558 436 D R	GCCACGTGGACTTTCTTTTTC		GGGTGATGTGAAGGAATTGG
436	E	1559 436 E F 1560 436 E R	TAGGAGACCCCTGTGGACAT		TGAGGCACAGAAAATCACITG
436	F	1561 436 F F 1562 436 F R	CTGCACCTCGAGGTGACAGAG		ACACCTGGCCACCACCTTACT
436	G	1563 436 G F 1564 436 G R	TCTCTGAGGTTTTCTGTCGCT		GGGATGAGCAGCAGAGACAC
436	H	1565 436 H F 1566 436 H R	CAGGTGCTGAGGAAAGGCT		TGCTGAGTGCTGGTCTTC
436	I	1567 436 I F 1568 436 I R	TGTGCCAGCTCCACTCTAAC		ATGTCAAATTTCCCTGCCCTG
436	J	1569 436 J F 1570 436 J R	GCCCCTGAGAAACACTTT		GGTCTGGAGAAAGGAAGGT
436	K	1571 436 K F 1572 436 K R	CCATTCCGGTAAAGATTCCA		ACACCCAGAGATGAGAGGC
436	L	1573 436 L F 1574 436 L R	CTACTTCAGTGCACCTTGGC		ATTCTCTGGGGTGATGTGG
436	M	1671 436 M F 1672 436 M R	CCATCAGTGTGCTGAGTGCT		ACAGGCCTCTTAAATTGCCA
449	A	1971 449 A F 1972 449 A R	CCAGATATCCAGCCTCAGC		ATCAGTGCCATCTCTGTCCC
449	A	1973 449 A F 1974 449 A R	CTGGGTAGAGCCCTGGCTAT		AAATGCTCCTGCCCTCAGAA
449	A	1975 449 A F 1976 449 A R	GGAAGAGGTGCTAGACGCTG		GCTAGGTGGATGGGTATT
449	B	1977 449 B F 1978 449 B R	AGTGGCCCTCAGGGTGAC		TCTCTGCTCCATCCTCAGGT
449	B	1979 449 B F 1980 449 B R	ATGTGGCAAAGCCAGBAC		CCCCAAGCATAGGACACAGA
449	C	1981 449 C F 1982 449 C R	TCAATCCCCAATCTCTTCT		CTCTTCCCTCTCCTTGCC
449	D	1983 449 D F 1984 449 D R	CAAGCCCATCTTACACAGA		TGTGGAGTGTGTAGTACTGGTCC
449	D	1985 449 D F 1986 449 D R	ACTGTGATGGACCTGCTCCT		TGTGTTGGTGTGGGAGGTC
449	E	1987 449 E F 1988 449 E R	CAAACATTATGAGCCTGGG		GTCGTTCTGACCCTTCAAGCC
449	F	1989 449 F F 1990 449 F R	TGTGGACTTAACACCTCTCCTC		TGAGTGTGGGAGAAGATCCC
449	F	1991 449 F F 1992 449 F R	GCTCCTTAGCCAAATATGGGA		ATAGATCCCCAGACCCCAACC
449	F	1993 449 F F 1994 449 F R	ATCCAAGGCCAAAGTCTG		TCTGGCCTGGGATAACTCAT
449	F	2011 449 F F 1992 449 F R	CAGGTGCTCTTAGCCAAATA		ATAGATCCCCAGACCCCAACC
515	A	1226 515 A F 1227 515 A R	GCTCCATCGGACTCACTAGC		TGGATTTCCAGGACTTTGAGG

515	A	1228 515 A F 1229 515 A R	TGTTGGGCTGGAGTTTATC	TCATGGCAACATGAAGAGC
515	A	1230 515 A F 1231 515 A R	GCGGTCGTGATGGACTACT	GCCATTCTGGATCAGCAACT
515	A	1232 515 A F 1233 515 A R	CAGCCAATCATCTCTGGCTT	CCACCATGATGAAGGTGATG
515	A	1234 515 A F 1235 515 A R	GCATCATCCTGTTCTGCTCA	TGATAAGAACGCCAGGTCC
515	A	1236 515 A F 1237 515 A R	GGCCATCGICTTTTGTCATCT	GCTGCTGCTCGGTTATTAT
515	A	1238 515 A F 1239 515 A R	ACTTCTCAGCCCCATCCTTT	GCAACAGCCCCAAGTGTCT
515	A	1240 515 A F 1241 515 A R	CATGGAGCCCTCTTATCTG	GCAACAGCTCTCCACTCAT
570	C	1310 570 C F 1311 570 C R	GGTTTCATCCTTGAAGACTGT	CCACAGAGGAAGACACAA
570	C	1312 570 C F 1313 570 C R	TAGCGGCATTCGCTATATT	ACCTTTCAACAGCCCAAGA
570	D	1314 570 D F 1315 570 D R	TGAGCTGGTTCTTACCTCCA	CAAGCCCAAGAAACAGGGA
570	D	1316 570 D F 1317 570 D R	AGGCATTGGAGTCTTTCAGC	AAATGGCCAAACAAAGTGCT
570	E	1318 570 E F 1319 570 E R	GAGAGCACAGTTGGTCCACA	ACAATGCTTTTGTGTCGGTG
570	F	1320 570 F F 1321 570 F R	CCTGATTGCGGGGAGTAAA	TCTGAATCCACAAGTGTGC
570	G	1322 570 G F 1323 570 G R	CGAAGTCTGTAGCCCAACATC	GTGCTGGACTCAGACACCT
570	H	1324 570 H F 1325 570 H R	CCATGTGTTAAAGTGCCCT	CCCCTCACTGGCTATTTTCA
570	I	1326 570 I F 1327 570 I R	GCTTGACATCACTGTGTTCC	AGAAAGGGAAGCTTGGGGTA
570	I	1516 570 I F 1517 570 I R	GGGAGCTCCTTGACAGACA	TGGAGCTGTTTTGTGTCATC
570	J	1330 570 J F 1331 570 J R	AAAATACCTGTAGCAGCGCA	ATTGGCTCTTGATCGCTGA
570	J	1332 570 J F 1333 570 J R	GCTACCCCTCCTGCTTTTCT	ATCAATCCAGGCAACATGC
570	B	1897 570 B F 1898 570 B R	TGCTGCTATTCTGGAAGGG	GCCGTGAGTTGAGCAGG
581	C	1362 581 C F 1363 581 C R	TCCGTGACTCTGGGATCTT	ATGAACCTCAACACCCCAAG
581	D	1364 581 D F 1365 581 D R	GGAAACCTTGCTTGGA	TGTTGGAACAGACCTGATTTTC
581	E	1366 581 E F 1367 581 E R	TGAGGGGAGAGATACAGGTGA	TGTTGCCACACACACAATG
581	E	1368 581 E F 1369 581 E R	ACAAGAATGTGCCTAACTGGC	GACTCCGCTTTGGGAAA
581	F	1370 581 F F 1371 581 F R	ACCATGCCCTTGCCAAAGAA	GCTCATACTGTGCTGCCAA
581	F	1524 581 F F 1525 581 F R	CAGTACTAGACATTTCTGCCAA	GGAATAAACAAAGCCAAACCG
581	G	1374 581 G F 1375 581 G R	GATTGTGGTTTGGCTGT	TCAGCATCCACAGATGAAG
698	A	1334 698 A F 1335 698 A R	GACCAGAATCCCAAGAGCAC	TGCTGTGATTGCCCTAACAA
698	B	1336 698 B F 1337 698 B R	TTTTGCCACTGAGATGCTA	AAATCCAGTGGCTTCTTCC
698	C	1338 698 C F 1339 698 C R	ACTGCTTTGTCTCCTGGAA	CACAAAACCTGAAACCCCTGCC
698	E	1342 698 E F 1343 698 E R	TGTTGGCTTGATCACTGAGA	TGACTGCCAAGCAATTTTCA
698	F	1344 698 F F 1345 698 F R	AGGAAGGTGTTTATGACGG	GCTCTTTCACCGAAAACCTGC
698	G	1520 698 G F 1521 698 G R	CAGGTGAGTTTAGTTTCTGTC	CCTCCCATCTTGACGTTTCA

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698	G	1522_698_G_F_1523_698_G_R	TCAGTTGCTGCTGTTGCA	AAACGGCATCTACCAATTAATC
698	H	1348_698_H_F_1349_698_H_R	CATCCCGTGAGTTGATT	CTCACTGCCACCCACAGTAG
698	I	1350_698_I_F_1351_698_I_R	TCCTGCTCCTCTGTAAGG	TTCTGGAAGACCCAGTTT
698	J	1352_698_J_F_1353_698_J_R	TGTGCTAGGCATGAATTG	CCCTCATCCTTTCATCTGTG
698	K	1354_698_K_F_1355_698_K_R	GGAGCATGTGAACACTGAA	GAACCCACCAAGGAGAA
698	L	1356_698_L_F_1357_698_L_R	AGTTTCAGACATCCGTGT	GCCTTTAAACCACAGCTATTG
698	M	1358_698_M_F_1359_698_M_R	TTGACTACAGCTGTGCCA	CTCTGGCCAAACAAGAAAGC
698	M	1360_698_M_F_1361_698_M_R	TCCTCCACTAAAGGTGCA	TCCTAATCCCTTCCCAAGT
698	D	1518_698_D_F_1519_698_D_R	TGTGCTCTCTGCTGTCTCT	ACCATTGTTATCCGGGCT
702	A	630_702_A_F_631_702_A_R	GGCCAGGGACATCAGGTT	GTCTGCAGCTGCCCTGTT
702	A	632_702_A_F_633_702_A_R	CCCCTCACCTGCTCTCT	CATAAGACGGGACTGTGCCT
702	B	634_702_B_F_635_702_B_R	AGTGAGCTGGGCTAGGCTCT	GGAGACCCCGTTCTCTCAC
702	C	636_702_C_F_637_702_C_R	CTGCTCCTCATCCTCACAGG	CCCTGAACCTTCCACGAGGT
702	C	638_702_C_F_639_702_C_R	GTCGAAGGGGTAGCCGTC	CCCTGTTCTCGTGACTCACTC
702	D	640_702_D_F_641_702_D_R	GGGGTTCTGACCCCTCTT	CAGTGGCTGTCCACGAGTT
702	D	642_702_D_F_643_702_D_R	ACCTTGCTCGTAGGGGAG	GCCCTTCTTGCCCTTAGTTC
702	E	644_702_E_F_645_702_E_R	CAGAGCCTGCTGCTGAGTG	GGACAGGGATGAGGACAGAC
702	F	646_702_F_F_647_702_F_R	CACACAAGGATGCCCTGTCC	GGCTGCACCCACAGAGTGG
702	G	648_702_G_F_649_702_G_R	TGGGTGCAGACCGTCTCT	CTCCATGAGGCGGACAGA
702	H	650_702_H_F_651_702_H_R	CTTGGCTGCCCTGTAGTGAT	CATCGACGCTGCCCTTCTC
702	H	652_702_H_F_653_702_H_R	CCTCGTGTGGTCACTCGTAAC	GGCTGACACAGGAGAAGGAA
702	I	654_702_I_F_655_702_I_R	CGAGGGTACCCACTCCCAT	ACCAACCCACCCACACT
702	I	656_702_I_F_657_702_I_R	AGCAGGGAGAGGTCAITTG	CAGAAGGGTGCCCACTCA
702	I	658_702_I_F_659_702_I_R	CCGAGATGCTCCCTCCAG	CACAGAGGGCAAGGACTGTG
702	I	660_702_I_F_661_702_I_R	TCGTAGTCAACACAGTCCC	CCAGGCCCTGACGCTATG
702	I	662_702_I_F_663_702_I_R	CACAGTCTTGCCTCTGTG	GCCCCTCCAGGACACAT
702	I	664_702_I_F_665_702_I_R	GTGCATGAGCAGACCTCGTA	TGCCTCCTACTTCTTCCGTG
702	I	666_702_I_F_667_702_I_R	CTCCACACACAGCCAGTC	CAGTCTTGTGCAAGCCCC
722	B	382_722_B_F_510_722_B_R	TTGAGTTCGCTATTGTGCC	GGACAGGTAGGCGGCTATG
722	C	813_722_C_F_814_722_C_R	GATTTGAGTTTGGCATGCTGT	ACAGCCAGAGGGACACACA
722	D	386_722_D_F_387_722_D_R	ATGTTGGATATTATAGCTCAGATGC	CAATATCCCATACTCCCAACATC
722	E	388_722_E_F_389_722_E_R	TTGAAAGTCAGGCTTGGACA	TTCAAGTCTGCAAGAAGAAAGT
722	F	390_722_F_F_391_722_F_R	ATGGCCCTCAGATACGAATG	TTGAAGTGAGACCTTAAGGGAGA

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722	G	512 722 G F 513 722 G R			ATGGTTGCAAAATGGCTTGT		ACAGAAGAGGACATGGAGCC
722	H	394 722 H F 395 722 H R			CCCTTTAACTTCCAACCCA		TCTTGGAGATGCAAGAGTCTG
722	I	396 722 I F 397 722 I R			CCATTACATGCACATCGTGT		TCTTGAAGGCCAAACTCACC
722	J	1526 722 J F 1527 722 J R			GCAATGCCATTGTTGATT		CGGGTTACAGCGTCTGAGAT
722	AA	739 722 AA F 740 722 AA R			TCAGCTTCTTCTTCTTGACA		GTGGCTGGCAAGCTTTTATT
722	A	1901 722 A F 1902 722 A R			GGGCTCCGCTGGAAAG		GGCCTGAACCGCTACCC
748	A	1985 748 A F 1986 748 A R			TAGCATCCACCTGTGGTCCC		CAGAAGCCAGAAAGGCAAG
748	A	1997 748 A F 1998 748 A R			GCTTCCATGGTTCCTTAA		TGCCTTCAATCAGTAGAAGAAC
748	A	1999 748 A F 2000 748 A R			TAAGATGGGTTGAGGGTG		TGGTTGAGAGAGCAAGAGGAA
751	U	1945 751 U F 1946 751 U R			GGTGCTACCTCTCTGATCCT		CACCTGCAGCCTCATGGTA
751	V	1947 751 V F 1948 751 V R			TAGCCTGTGGTGAGGGCAGT		TCCTGTGACCTCAAAGCATCC
751	W	1949 751 W F 1950 751 W R			TGCCACTCAGGGTGACTGT		TGCAAGCCTGCTCCTGAT
751	X	1951 751 X F 1952 751 X R			CCTAACTACGTGCAAGGGC		GCTCAGGATTTGAGTCCCAG
751	Y	1953 751 Y F 1954 751 Y R			ATTTCCAAATCCCAACCTCC		CTGGGACCTCGGTTTATG
751	Z	1955 751 Z F 1956 751 Z R			TCACTGGGCTTATGGCTCTC		GTCCATGAGCAAAAGGTGGAG
848	Y	2001 848 Y F 2002 848 Y R			GCCTCCAACCTTTGCCCTCTC		TAAACGGCAATCCACCTC
848	Y	2003 848 Y F 2004 848 Y R			TCCTCTCGCCCTCTCTCTG		TAAACGGCAATCCACCTC
848	Z	2004 848 Z F 2005 848 Z R			CATTGTCTTCACTGGCCG		TGGTGTCTGCCGCTGATT
GenR2	A	1453 GenR2 A F 1454 GenR2 A R			CCAAGCCCCCAAAATTTAAGTG		CCTCTGCGCTAAAACTGTGC
GenR2	B	1455 GenR2 B F 1456 GenR2 B R			CATTCTTGGCACACAATGG		TGGTTGAGCCACCATACTCA
GenR2	C	1457 GenR2 C F 1458 GenR2 C R			TATTTACCCAGGAGGTTTCG		TGTTGCCAAGATGTGAAA
GenR2	D	1459 GenR2 D F 1460 GenR2 D R			TCCTCCTAGGAACAGAGCCA		ATGCACTCAGCGACCTTCTC
GenR2	F	1575 GenR2 F F 1576 GenR2 F R			GTCTTCCCATCCCTCAACA		GGGAGGCATAATGAACCAGA
GenR2	F	1577 GenR2 F F 1578 GenR2 F R			TAGCGCCCTATCCCTTCTT		TCCATCCCAAGCTTCACCT
GenR2	E	1790 GenR2 E F 1791 GenR2 E R			CTCTGACCTTGCACCTACCCC		CCACCGTGTCTTCAAATCA

- 5 Comparative DNA sequencing was used to determine the sequence changes in the genes in 12q23-qter. Variants detected by SSCP analysis in the initial set of asthmatic and normal individuals were analyzed by fluorescent sequencing on an ABI 377 automated sequencer (Perkin-Elmer Applied Biosystems Division). Sequencing was performed using Amersham
- 10 Energy Transfer Dye Primer chemistry (Amersham-Pharmacia Biotech) following the standard protocol described by the manufacturer. Primers used for dye primer sequencing are shown in Table 9. Column 1 lists the genes targeted for sequencing. Column 2 lists the specific exons sequenced. Columns 3 and 4 list the forward primer names and the forward
- 15 primer sequences, respectively. Columns 5 and 6 list the reverse primer names and reverse primer sequences, respectively.

TABLE 9: SEQUENCING PRIMERS

Gene	Exon	Forward Primer	Forward Sequence	SEQ ID NO:	Reverse Primer	Reverse Sequence	SEQ ID NO:
454	B	MDSseq_118_454_B_F	CCAGATACTGGGCAAGGAG		MDSseq_118_454_B_R	GCACCAGGACATGAGGCTAT	
454	E	MDSseq_119_454_E_F	AGCCAGCAGAAATCCACAGTC		MDSseq_119_454_E_R	GGTACCCCTGGAAAGATCTGGG	
454	E	MDSseq_473_454_E_F	TCCTGTTACTCTCTGCGGT		MDSseq_473_454_E_R	CCAACCTACGCAAGAAATGA	
454	F	MDSseq_120_454_F_F	ACAGCAAGGAGGAAGTCCG		MDSseq_120_454_F_R	TGGAAAAGGGTTCTCCAGC	
454	G	MDSseq_121_454_G_F	TTCTCCAGAGCAAGTGACC		MDSseq_121_454_G_R	CCACAGGAAAGGAATACACCA	
454	H	MDSseq_122_454_H_F	AGTGCCCTGAATCCAGTCT		MDSseq_122_454_H_R	CATTCATCTTGTGCTTGG	
454	H	MDSseq_291_454_H_F	AGTGCCCTGAATCCAGTCT		MDSseq_291_454_H_R	CATTCATCTTGTGCTTGG	
454	K	MDSseq_123_454_K_F	CCCAGAACCCAGCACTTC		MDSseq_123_454_K_R	TAGAAATTGCTTCCAGGCC	
454	L	MDSseq_124_454_L_F	GTCTCCCTTAATGTGGG		MDSseq_124_454_L_R	GGGCCTAAATTTCTGTCAT	
454	M	MDSseq_125_454_M_F	CCAGCACITTAAGCATCTA		MDSseq_125_454_M_R	CTTCCCTCTATCTTGCCCT	
454	N	MDSseq_126_454_N_F	AGCATGGGGTTCCCATTT		MDSseq_126_454_N_R	ATTGGAAGGGGGCATAAAAG	
454	O	MDSseq_127_454_O_F	CGATTCTCTGGACAAACCAGA		MDSseq_127_454_O_R	GGACAGTTTGTCTGTGCCTC	
454	O	MDSseq_128_454_O_F	GAACACATGCATGGTCTGA		MDSseq_128_454_O_R	ACAGACAGGATTTCGCCCTTG	
454	AA	MDSseq_460_454_AA_F	CTCAACTCCACTTTCTCTCGG		MDSseq_460_454_AA_R	CAAGAAGCGCCCAAGTCTCTAC	
454	AA	MDSseq_470_454_AA_F	TGCATCTTTGAGTGACTGCTG		MDSseq_470_454_AA_R	ACTCTGGTCTGCAGTTGGTG	
454	AA	MDSseq_471_454_AA_F	TCTTGACATTTGCAAGGC		MDSseq_471_454_AA_R	TCAGAATGTGCACCTGAAGC	
757	A	MDSseq_407_757_A_F	CTCGCTTCCCGTATTGTT		MDSseq_407_757_A_R	GCCTCCATGCACAGGTAGTT	
757	A	MDSseq_408_757_A_F	TTCTTCTGTGCTCGCTGTA		MDSseq_408_757_A_R	CTCTCCAGTCCCTCCTGGAT	
757	A	MDSseq_409_757_A_F	CGTGGACGTGTACTGGAGC		MDSseq_409_757_A_R	CTCCAGCTTGTCCGTGTTCT	
757	A	MDSseq_410_757_A_F	AGCCAAACAGCAGCTACTTCC		MDSseq_410_757_A_R	GACTGGGCGAGGATCTCATA	
757	A	MDSseq_411_757_A_F	TCITTATGCTGCTGGTGGTG		MDSseq_411_757_A_R	GGGCTGTCTTCTCTCTGC	
757	A	MDSseq_412_757_A_F	AGGGAAGCTCCTCCAGTGA		MDSseq_412_757_A_R	TCTGCCAACCTAGTGTCTCC	
757	A	MDSseq_413_757_A_F	TGAACCTCAACGATGTGCAA		MDSseq_413_757_A_R	TTCCAACITTCACACATTGCC	
757	A	MDSseq_418_757_A_F	CTCGCTTCCCGGTATTGTT		MDSseq_418_757_A_R	GCCTCCATGCACAGGTAGTT	
757	A	MDSseq_419_757_A_F	AGGGAAGCTCCTCCAGTGA		MDSseq_419_757_A_R	TCTGCCAACCTAGTGTCTCC	
757	A	MDSseq_421_757_A_F	CAAACITTTGCTGCTCTCCG		MDSseq_421_757_A_R	AGTTGGGGTCTGTTCTTGTTG	

757	A	MDSeq_422_757_A_F	CAAGAAAGAGGCCGAAGTTTG		MDSeq_422_757_A_R	TACAGCGAGCACAGGAAGAA
757	A	MDSeq_423_757_A_F	GAGGACACGTCCAACGCC		MDSeq_423_757_A_R	CTCGTCCGAGCCGTTGTT
757	A	MDSeq_424_757_A_F	CAAGAAAGAGGCCGAAGTTTG		MDSeq_424_757_A_R	TACAGCGAGCACAGGAAGAA
757	A	MDSeq_425_757_A_F	GAGGACACGTCCAACGCC		MDSeq_425_757_A_R	CTCGTCCGAGCCGTTGTT
561	B	MDSeq_169_561_B_F	ACTGCTCTCCCGTGAAAGTG		MDSeq_169_561_B_R	CCATCAGCATCTGTGTGACC
561	C	MDSeq_170_561_C_F	TTAAGCCAAAGGAAAGGAGCA		MDSeq_170_561_C_R	CCTCGATGGGATTGTCTTT
561	E	MDSeq_171_561_E_F	ATCTGTGTGTGAGCTGGC		MDSeq_171_561_E_R	GGGTGCTGAAAGACAAGAGC
561	H	MDSeq_172_561_H_F	AAATGGTTGACGTCACTGGC		MDSeq_172_561_H_R	CTGTGGCTGTGGCAGGAT
561	J	MDSeq_173_561_J_F	TGTTGGAGCTGAGAGACCTG		MDSeq_173_561_J_R	CCTCTAAACTCTCTTTATCCAGACC
561	H	MDSeq_174_561_H_F	CTGTGGGCAGGAGACTGGT		MDSeq_174_561_H_R	TGACAGAGTCCACACAGCAA
561	M	MDSeq_177_561_M_F	ACCTGCCCTGATGAGAAGAA		MDSeq_177_561_M_R	TGTTTGCAAGCAAGACGGTA
561	P	MDSeq_183_561_P_F	AGGCAGATTCTCAGCTCCT		MDSeq_183_561_P_R	CAGAGGGCAATAAACCTCCA
561	G	MDSeq_390_561_G_F	GCATTTCCAGAAGATGGTG		MDSeq_390_561_G_R	TAATCCAGAGCAGACGAGGG
561	H	MDSeq_392_561_H_F	CTCTGGGCAGAGGACTGGT		MDSeq_392_561_H_R	TGACAGAGTCCACACAGCAA
561	X	MDSeq_401_561_X_F	GAACTGCCCTGTCCATCTGT		MDSeq_401_561_X_R	AAATCTCAGGCTGGGAGGAC
561	Y	MDSeq_402_561_Y_F	ACAACCTCCAAATGGCGAGAA		MDSeq_402_561_Y_R	CCAAGCAGAGATAACCAGCA
561	X	MDSeq_415_561_X_F	GAACTGCCCTGTCCATCTGT		MDSeq_415_561_X_R	AAATCTCAGGCTGGGAGGAC
561	X	MDSeq_417_561_X_F	GAACTGCCCTGTCCATCTGT		MDSeq_417_561_X_R	AAATCTCAGGCTGGGAGGAC
214	B	MDSeq_15_214_B_F	GACAGTCTGCTCCACATCCA		MDSeq_15_214_B_R	TGGAGATGAAGTCTTGCTCT
214	C	MDSeq_110_214_C_F	ATATGTTTGCTGGCTTTGGG		MDSeq_110_214_C_R	CCCAGGCTGTGTGCTCTCTA
214	E	MDSeq_343_214_E_F	TGCTTCCTGTTTGTCACTGC		MDSeq_343_214_E_R	TGAGGACACGATGAACCTGA
214	E	MDSeq_383_214_E_F	ATGACCTGGGTGAGGACTT		MDSeq_383_214_E_R	GCAGTGACAACACAGGAAGCA
214	AA	MDSeq_399_214_AA_F	CGAATAAAGGCGTCGAGAAG		MDSeq_399_214_AA_R	CCTTCTGGAGAGGACGTG
224	BB	MDSeq_403_224_BB_F	AATTGACTTCCCGCCTTCT		MDSeq_403_224_BB_R	GCCCAGCATCCTTCTACTT
422	E	MDSeq_431_422_E_F	AAGCATCTTGGCGAAGTCAT		MDSeq_431_422_E_R	AAAGGAGACACTGCCAGAA
422	F	MDSeq_434_422_F_F	TGGGCATCCTGATGTACTTG		MDSeq_434_422_F_R	GTGGTGATGCCTATGGTC
422	C	MDSeq_323_436_C_F	TGTGAAAAGTGTGTGCTGAA		MDSeq_323_436_C_R	AGTTTGGGTGACAGAGCG
422	D	MDSeq_324_436_D_F	TGTGAAAAGTGTGTGCTGAA		MDSeq_324_436_D_R	AGTTTGGGTGACAGAGCG
422	E	MDSeq_325_436_E_F	TCITTAGCTTGGCATCACCC		MDSeq_325_436_E_R	ACGCAGAGTTGAAGGTGCTT
422	G	MDSeq_326_436_G_F	CTGCACCTCGAGGTGACAGAG		MDSeq_326_436_G_R	AGCCAGGAGATACGTTGTGC
422	K	MDSeq_327_436_K_F	GCTAGGCATGGTGAGTGGTT		MDSeq_327_436_K_R	CGCAAGGTGCACCTGAAGTAG
422	R	MDSeq_340_436_R_F	CCATCAGTGTGCTGAGTGCT		MDSeq_340_436_R_R	ACCCAAAATGTGAAAAGGTG

422	L	MDSseq_374_436_L_F	GCACAGGCCCTCTCATCTCT			MDSseq_374_436_L_R	AGAGTTGACCCAGCCCAAGAA
422	A	MDSseq_375_436_A_F	CAAGATTCCTCTCACCTCGG			MDSseq_375_436_A_R	AACAGCAGCAAGCAGCCT
422	C	MDSseq_393_436_C_F	TCACTGTTTCCATTGGGTTA			MDSseq_393_436_C_R	GTAGGGCAAGAGCTGGGATG
422	D	MDSseq_394_436_D_F	TCACTGTTTCCATTGGGTTA			MDSseq_394_436_D_R	GTAGGGCAAGAGCTGGGATG
422	G	MDSseq_395_436_G_F	GGCTGCAGAAAACCTCACCTCT			T MDSseq_395_436_G_R	TGAGTGTGGTCTTCAGTGG
422	A	MDSseq_396_436_A_F	GCTGGGATGACAGGTGTGAG			MDSseq_396_436_A_R	TCCCAAAGTGTCTGGATTAC
422	A	MDSseq_404_436_A_F	AGGAGCCTTTTCGCTCTCAA			MDSseq_404_436_A_R	ATGTTGCCCAAAATGGTTTC
422	D	MDSseq_414_436_D_F	TCACTGTTTCCATTGGGTTA			MDSseq_414_436_D_R	GTAGGGCAAGAGCTGGGATG
422	D	MDSseq_416_436_D_F	TCACTGTTTCCATTGGGTTA			MDSseq_416_436_D_R	GTAGGGCAAGAGCTGGGATG
436	C	MDSseq_323_436_C_F	TGTGAAAAGTGTGCTCTGAA			MDSseq_323_436_C_R	AGTTTGGGTGACAGAGCG
436	D	MDSseq_324_436_D_F	TGTGAAAAGTGTGCTCTGAA			MDSseq_324_436_D_R	AGTTTGGGTGACAGAGCG
436	E	MDSseq_325_436_E_F	TCITTAGCTTGGCATCACCC			MDSseq_325_436_E_R	ACGCAGAGTTGAAGGTGCTT
436	K	MDSseq_327_436_K_F	GCTAGGCATGGTGAAGTGGTT			MDSseq_327_436_K_R	CGCAAGGTGCACCTGAAGTAG
436	B	MDSseq_340_436_B_F	CCATCAGTGTGCTGAGTGCT			MDSseq_340_436_B_R	ACCCAAATGTGGAAGGTG
436	L	MDSseq_374_436_L_F	GCACAGGCCCTCTCATCTCT			MDSseq_374_436_L_R	AGAGTTGACCCAGCCCAAGAA
436	C	MDSseq_393_436_C_F	TCACTGTTTCCATTGGGTTA			MDSseq_393_436_C_R	GTAGGGCAAGAGCTGGGATG
436	D	MDSseq_394_436_D_F	TCACTGTTTCCATTGGGTTA			MDSseq_394_436_D_R	GTAGGGCAAGAGCTGGGATG
436	G	MDSseq_395_436_G_F	GGCTGCAGAAAACCTCACCTCT			T MDSseq_395_436_G_R	TGAGTGTGGTCTTCAGTGG
436	A	MDSseq_404_436_A_F	AGGAGCCTTTTCGCTCTCAA			MDSseq_404_436_A_R	ATGTTGCCCAAAATGGTTTC
436	D	MDSseq_414_436_D_F	TCACTGTTTCCATTGGGTTA			MDSseq_414_436_D_R	GTAGGGCAAGAGCTGGGATG
436	D	MDSseq_416_436_D_F	TCACTGTTTCCATTGGGTTA			MDSseq_416_436_D_R	GTAGGGCAAGAGCTGGGATG
449	D	MDSseq_462_449_D_F	GTCACACAGCCAGTAGGCAG			MDSseq_462_449_D_R	CAGAGAGCAAGAAGGCCAAG
449	F	MDSseq_463_449_F_F	AAGAGAAAATCCGGAGGACC			MDSseq_463_449_F_R	ACGGGGTCTCCCTGTGATA
449	A	MDSseq_472_449_A_F	CCAACCTCAGTTTCCCAACG			MDSseq_472_449_A_R	CAGGGACGTGGACTCTGATA
449	F	MDSseq_474_449_F_F	CACATATCTGCCTGCTCCT			MDSseq_474_449_F_R	CACCATCAGGATTCTTCACG
515	A	MDSseq_235_515_A_F	CAGCCATCATCTCTTGCCCT			MDSseq_235_515_A_R	ATTACTCGATGCCAACAGCCC
515	A	MDSseq_236_515_A_F	TGGACCTGGCGTTCTTTATC			MDSseq_236_515_A_R	CAGGAGCAACACAAATTCCTT
515	A	MDSseq_237_515_A_F	CGTAGTTTCTCGGTAACCAATCA			MDSseq_237_515_A_R	TTGGAGATCTTGTTCAGGGC
515	A	MDSseq_239_515_A_F	GGCCATCGTCTTTGTCATCT			MDSseq_239_515_A_R	GCGTCAGAGATGAAGCAAGT
515	A	MDSseq_263_515_A_F	CTGCTGTGTGTCCGAGATG			MDSseq_263_515_A_R	GTTGTCAGGAGCCAGCAAGT
515	A	MDSseq_265_515_A_F	GGCCATCGTCTTTGTCATCT			MDSseq_265_515_A_R	GCGTCAGAGATGAAGCAAGT
570	C	MDSseq_266_570_C_F	TTGATGTGTTCGCTCTCT			MDSseq_266_570_C_R	GCATGAGCTCTGGAAATCAGG.

570	F	MDSeq_268_570_F_F	CACCTGATTATTTCCCTCA		MDSeq_268_570_F_R	AACCTCCCTTTAACTCAGTC
570	I	MDSeq_270_570_I_F	CTGAGTGAGCGGAGGTGTT		MDSeq_270_570_I_R	TTGGCAATTTCTTTTCATCAG
570	J	MDSeq_271_570_J_F	CAGACAGCCACCTCCAG		MDSeq_271_570_J_R	CCAAGACTTTGCAATCTCCA
570	I	MDSeq_294_570_I_F	GCTGGCACTGGTGTATCA		MDSeq_294_570_I_R	CCACGTAGGAATGGAGCTGT
581	E	MDSeq_277_581_E_F	GGGAGATTGATAGGTCAGC		MDSeq_277_581_E_R	TAGCCAGGCGTGGTGTA
581	F	MDSeq_345_581_F_F	CCITCTGAGTAGCTGGGCTC		MDSeq_345_581_F_R	TAGACTTCTGACGCTGGGCT
698	B	MDSeq_274_698_B_F	TGTCCTGGACCACACAGTT		MDSeq_274_698_B_R	CGGCTAAGTCTTTTCATCAG
698	E	MDSeq_275_698_E_F	GTAAGCATTTGTGTGGCAGC		MDSeq_275_698_E_R	TGCCAAGGGCTGTTTCTAAT
698	H	MDSeq_280_698_H_F	TGTGTACAGATTGCCCTACCC		MDSeq_280_698_H_R	TGACGAATACAGGATGAAAGTC
698	I	MDSeq_287_698_I_F	GACAGCGCTCTGGGTATTA		MDSeq_287_698_I_R	TGAAACAGGCCAGAGAAGTTT
702	C	MDSeq_111_702_C_F	GTGATGAGGACAAGCTCGG		MDSeq_111_702_C_R	ACGTTCCACGGGACTCA
702	D	MDSeq_112_702_D_F	CAACCTGCCTGTCGTAAC		MDSeq_112_702_D_R	CGCTCCATGAATGTACAAA
702	A	MDSeq_113_702_A_F	TCCACCACTCTCCTGCG		MDSeq_113_702_A_R	AAGGTGGAGGCCCTGAC
702	B	MDSeq_114_702_B_F	CCCTCTGATCAGGCACAGTC		MDSeq_114_702_B_R	GGATATCTACAGCAGGCCCA
702	F	MDSeq_115_702_F_F	ACGCTTCTTGTAGGACCGAA		MDSeq_115_702_F_R	AAGACGATCTTGTGTCGCT
702	I	MDSeq_116_702_I_F	AGCAGGGAGAGGTGATGTTG		MDSeq_116_702_I_R	GGTGTGTGGAGACTCACAGG
702	I	MDSeq_117_702_I_F	CACTAGGGAGACAGTCCGT		MDSeq_117_702_I_R	CTGCCATCTAGCACAGGCC
702	B	MDSeq_178_702_B_F	AGGCACAGTCCCGTCTATG		MDSeq_178_702_B_R	GAGAGCTCTGCTGCTGCT
702	I	MDSeq_179_702_I_F	TCGTACAGTCAACACAGTCCC		MDSeq_179_702_I_R	CCCACCTGCAGTCTTGTGC
702	C	MDSeq_191_702_C_F	AGATCGGCTAGTGGGAAAT		MDSeq_191_702_C_R	GCTCTCATTTCCCTCCCTC
702	I	MDSeq_196_702_I_F	CAGTCTTGTGCAAGCCCC		MDSeq_196_702_I_R	CACAGTCTTGGAGACTCACAGG
702	I	MDSeq_269_702_I_F	AGCAGGGAGAGGTGATGTTG		MDSeq_269_702_I_R	GGTGTGTGGAGACTCACAGG
722	F	MDSeq_63_722_F_F	TAAGTAGGTTGTGACCGGC		MDSeq_63_722_F_R	CACTCTCCCAATCTCCCTGA
722	C	MDSeq_132_722_C_F	ACCTGATAGGTTTCCCGGT		MDSeq_132_722_C_R	ATACAGATGCCCTGGCTCG
722	AA	MDSeq_135_722_AA_F	GACACGATCTCGCTCTCTG		MDSeq_135_722_AA_R	GCCTGGGTGACACAGCTA
722	B	MDSeq_141_722_B_F	TTCAGCCAGGATCTGTTGTG		MDSeq_141_722_B_R	GGGCTGGGAGTTACCTTAT
722	B	MDSeq_146_722_B_F	TGCAACACACGACGTTTAC		MDSeq_146_722_B_R	ACCTCTACGGCAGGCTGAAT
722	G	MDSeq_150_722_G_F	CAGTGTGCGGAGACATTGTT		MDSeq_150_722_G_R	TGAGTCTCCACAACATAGC
722	A	MDSeq_441_722_A_F	TATTACCCAAAGCTGCACCC		MDSeq_441_722_A_R	TCAGGACTCCCTGAGACCC
751	U	MDSeq_455_751_U_F	AGACACTCTCCAGCTCTCGC		MDSeq_455_751_U_R	GCAGGACCCCTGGACTACAGA
751	W	MDSeq_456_751_W_F	CTCCAGGTAATGCTCAA		MDSeq_455_751_W_R	TACTGTCTCTCCATTCCCAGC
GenR2	F	MDSeq_420_GenR2_F_F	CCAGGAGACAGAGGTTTCA		MDSeq_420_GenR2_F_R	CCCAGACTGGCTTTGAAGTC

Single nucleotide polymorphisms (SNPs) that were identified in genes from the disorder region are shown in Table 10. Column 1 lists the gene names. Column 2 lists the exons that either contain the SNPs or are flanked by intronic sequences that contain the SNPs. Column 3 lists the PMP sites for the SNPs. Column 4 lists the localization of the SNPs to exon, intron, or UTR sequences. Column 5 lists the SNP reference sequences and illustrates the SNP nucleotide changes with underlining. Column 6 lists the SEQ ID NOs of the SNP reference sequences. Column 7 lists the base changes of the SNP sequences. Column 8 lists the amino acid changes resulting from the SNP sequences.

The "-" symbols denote polymorphisms which are 5' of the exon and are within the intronic region. The "-" polymorphisms are numbered going from the 3' to 5' direction. The "+" symbols denote polymorphisms which are 3' of the exon and are within the intronic region. The "+" polymorphisms are numbered going from the 5' to 3' direction. The first, second, and third columns, combined, correspond to the SNP names as described herein, e.g., 214_B_1, 214_E_+2, etc. It should be noted that the disclosed SNPs are referred to herein using both short (e.g., 757_A_+4) and long (e.g., Gene 757 A +4) nomenclature.

The genomic sequences corresponding to the genes in Table 10 are shown in Tables 3A and 3B. Taking the information from Tables 3A and 3B, in combination with the last column in Table 4, one of skill in the art could identify the entire genomic sequence of the genes and SNPs described below. For example, the genomic sequence for Gene 214 is contained within BAC clones RP11-702C13 and AC079031 (see Table 4), and the nucleotide sequence of BAC clone RP11-702C13 corresponds to SEQ ID NO:766 to SEQ ID NO:808.

TABLE 10: SNPs

Gene	Exon	PMP Site	Location	Sequences	SEQ ID NO	PMP	AA change
214	B	1	3' UTR	CCTGTGCACCTCTGGGCATACGCTAGGAGTGGAACTGCTG		C>T	
214	C	-1	Intron	GGGCTCTGGCCACCTCAACCCAGGCGTTGTTCGCAGGA		C>T	
214	E	+1	Intron	AAGGACACATCTTATCAGCTGTAGTACCACGTTTCATTAC		T>C	
214	E	+2	Intron	CCCTGTGACGCTCAACTCCCGTCCCTCCAGCCCTGACAG		G>C	
214	E	+3	Intron	CTCAACTCCCGGTCCCTC**CAGCCCTGACAGCCACTGTT		**>TC	
214	E	-1	Intron	AGGCCGCTTCAACCTTCTCTCGGCGAGGGGCAATGGCCAA		C>T	
214	E	1	Exon	CACCTGCATTCCCTCTCTCTGTGAGTGTCTGGGGCCCGTT		G>T	Val > Leu
214	E	2	3' UTR	GGGCTCTGGCCACCTCAACCCAGGCGTTGTTCGCAGGA		C>T	
214	E	3	3' UTR	TCAGGAGCCTGTGCTTGACCCCAATCCGCCCCCAACTC		C>T	Pro > Ser
422	E	1	Exon	CAGACACATGACAACTGCTATGACAGGCCAAGAGCTGGA		T>C	
422	E	2	Exon	ACCCACACCTATTCACTACTGCTCTGGCTGGCAATCAC		G>A	
436	A	+1	Intron	GGCCGCGCGGGGCGGCGGCGGTGCTGCCCTCGCGTCCGC		G>T	
436	A	+2	Intron	CTGCTTGTGCTGTTTAAAGCCACAGCCTGGGCCAGGCGC		G>A	
436	A	-1	Intron	CCTTCCGGGCCATCATCCGCGATGACGGCGCCGCCAGCAGG		G>T	
436	A	-2	Intron	GCCCTCCCCCGGGCCCGGG*****CCCGACCCCGCGT		*****>CCCCGGG	
436	A	-3	Intron	TCCTCAAGGGMGAGGCCACTCCCGCCCCCGGAGTTCCAT		CC>**	
436	A	1	Exon	CGGGCGCGCGGCCATGGCGGCTGCTGCGCCGCGCTGGCG		G>T	Gly > Cys
436	A	2	Exon	TGAACCGCGCGTGCAACTGCTCATCTCGCCTACGTCATC		C>T	Leu > Phe
436	C	+1	Intron	ATTCCAGATCGGACCACCTGTGTAAATCAGATGCCAGCTG		G>A	
436	C	+2	Intron	AACGGTACGAGCTTGTGGCTCTCTGGGAGGGCAGCCCTG		T>C	
436	C	+3	Intron	TGTGGCCTCTGGGAGGGCAGCCCTGAGCAGATCGCCCC		A>G	
436	C	-1	Intron	CTCTCCGTGAGTCTCTGAGCGTGGCTTGCCTGCTGCT		C>T	
436	D	-1	Intron	GTCCACCTGTGTGTGGGCGGGCCACGTGGACTTCTTT		G>A	
436	D	1	Exon	ATTCCAGATCGGACCACCTGTGTAAATCAGATGCCAGCTG		G>A	
436	E	1	Exon	TGGTAGCTTTCAACGGGTCTGTCAGACGCTGTGAGGTGGC		C>T	
436	G	1	Exon	TAGTGGAGAACGGAGGACACAGTTTCCAGGACATGGCCGTG		A>G	Ser > Gly
436	K	+1	Intron	GGGAGGCCCTTCTGCAGAGGCTGGCACCAGTGTGGCGTGGT		C>G	

436	K	+2	Intron	RCGACATCTCARGTTGGTGAATGATATGCATGCTCTGAGAA	T>A	
436	K	-1	Intron	GCTCACTCTCACCTATGCTAACTCAGGCGACCGTGCTGT	A>G	
436	K	-2	Intron	GATTCCAGGCTTCTCAGGAAGGGCAGCAAAAGATAAGAT	G>C	
436	L	-1	Intron	CCAGGAGGCACCTCCCTCCGGCTGCCACAAAGGGTCCCA	C>T	
436	L	-2	Intron	GGTGAAGTCCCAGGAGGCAGCTCCCTCCGGCTGCCACAA	C>A	
436	L	-3	Intron	TGGCGTGTGTCCCGTTAAGCCGGGCGAGTCTGCCACTCT	C>T	
436	L	1	3' UTR	AGCAGCTCTGTGTGTGTGTCAGGATCTGTTGCCCACT	T>C	
454	B	-1	Intron	AAGTGCCTGCATCTCCAAAGCTGCATCCCAACCCGCTGT	C>T	Val > Ala
454	B	1	Exon	AGAGGTAAAGAGGAGATCGTGGAGATGGATGAAGAAGT	T>C	
454	E	-1	Intron	CTCCTGGAGAACGTCTCTCCGAGTCTTTCACATCTGTG	C>T	
454	E	-2	Intron	CAAAGCTAGTCTCTCGCCCGGGTTGAGTTAATGATGTCCC	G>A	
454	E	1	Exon	CCCCTATAGGAATTCAGACCCGGAAGGTGTGTAGTGTATGAA	G>A	Gly > Arg
454	E	2	Exon	AGACCGGAAGGTGTGTAGTGCATGAAGGGAACCAAGAGACC	C>T	His > Tyr
454	E	3	Exon	TGTGAAGTCTCTGCTGGTGGTCCCATCGAGGAGTGAAGA	C>T	
454	F	+1	Intron	TCTACTGCTGAGTAATAATATCCCAACCTCAGAAAGCCT	T>C	
454	F	-1	Intron	CATGGCTCCCTCGGTCCCAACCCGTCACATAATGGCCATTTT	A>C	
454	F	-2	Intron	GGCCCATGGGCTCCCTCGGTCCCAACCCGTCACATAATGGCCA	C>T	
454	F	-3	Intron	TGTATCCATTCTCTCATGTCATCCCAAGACCAAGCCCAAG	C>T	
454	G	-1	Intron	CACCTCTGGGAAAGAGACAGATCTGTTTCAATCGAGATGT	A>T	
454	H	-1	Intron	GTTCTTCAATCAGCATTTTCTCTATAAAACCTTAAGCAAT	C>T	
454	H	-2	Intron	TTTAGGACAAATGAGTTAAGCGTGATGTGCCAGACGGGG	G>A	Arg > His
454	H	1	Exon	CCGTTGGTTCCATCACTGCCGTCCTCAAAATACAGTTTCCGTC	G>A	Arg > His
454	H	2	Exon	CCGTCCCAATACAGTTCCGTCGCTTGACGACAAAGACCA	G>A	Arg > Gln
454	K	1	Exon	GGAAACAATGTTGAGAAACGGACTCTGATAAAAGTCTTCG	C>T	
454	L	-1	Intron	ATTACCTGAGTAAACTCTCCACTCTGTTTATAGGGAGG	C>G	Thr > Ser
454	M	2	Exon	CATCGACTTCTCATCGACACTTACTCCAGTAACCTGCTGTC	C>T	
454	M	+1	Intron	GTTTCACAGGACCAAGACAGCGGAGAGATCCATGAAATCA	C>T	
454	M	+2	Intron	GTAGTGGATAGTCGCTGGGCTCTACCCCGATCAACCAACT	A>G	Thr > Ala
454	M	1	Exon	GTCTGCATTCTCCCAAGGCCACTGTGTTTCATCGACTTCTCTC	G>A	
454	O	+1	Intron	CTCACGCTCTGTAATCCCAAGCGCTTTGGAGGCCGAGGAGG	T>C	
454	O	-1	Intron	ATAAATCATGTAATATTAAATGTAAGTTAATAA	A>G	Gln > Arg
454	O	1	Exon	TGGACAACCAAGAGGAGATACAGCTGCTTAGAAAGGAGGCGA		

454	O	2	Exon	CCTAGATCAGGGATAGCCCGTCTGGTGCCAGTGTGGAAG	C>T	Glu > Ala
454	O	3	Exon	GAGCACAGGTGCTGGAGGAGCTGTGCTGCCGAAAAGC	A>C	
454	O	4	Exon	CTCTACCAGAGCCCTTGTGGCTGGATGTGGATCCAC	G>T	
454	O	5	Exon	GGACATGGCTGACTTGGCACTCTGCCAGCTGTGCCGCT	T>A	Ile > Asn
454	O	6	Exon	AGGATCCGGAAGAGTTTCCAAAGAGTGAAGGGCAGTACAG	A>G	
515	A	1	Exon	CAGCGTGGTTGTGCGGATCCGATCTTCTGGCTCTGCACA	G>A	Arg > His
515	A	2	Exon	CGCTGCCTCCAGAGGAAGATACAGGTGAGCCAGATAATAA	G>A	Met > Ile
515	A	3	Exon	GGCGCTCCAGAGGCGTTAATGGCCAACTCCGGTGAGCCATG	G>C	MET > Ile
515	A	4	Exon	GTCACCTGACTCGGCCCTAAGGTTTCTCTGGAACCTCCAGATT	G>A	Val > Ile
515	A	5	Exon	ACTCCAGATTCAGAGAATGTGATTAGGGAAACTGTGGCA	C>G	Ile > MET
515	A	6	3' UTR	CTCCAGATTCAGAGAATCTGATTAGGGAAACTGTGGCAG	T>C	
515	A	7	Exon	CTGGTTCAAGGTGTGACCAAGGAATCCTGGAGGAACAGA	A>G	
561	B	+1	Intron	TGTGGTGGGAGAGAATGGCGTTGGCTGCCTCGAGGGTG	C>T	
561	B	+2	Intron	CGAGGTGTGCACAGGTGAATCGGTTTGGTGACACCTGGC	A>G	
561	B	1	Exon	AAGTCCGGCAGCAGCTGGCAAGATTGACCTGCTGGGTGG	C>G	
561	C	1	Exon	GAATATATCGGGCCCTTCCGAGCCTGGTGACAGGCCGGA	G>A	
561	E	+1	Intron	CAGGCTCCCAACATACCTGGCCACCCAGCCCTCTCTCTC	T>C	
561	E	+2	Intron	ACTCGTAGTTACCAGGTTTCCCTCTTTGACGACTGGAAA	G>C	
561	E	1	Exon	AGCTGAGCTGCCCTCAGCGGGGGGAAAATACCTCTACGTCT	C>T	Ala > Val
561	G	+2	Intron	GGGTGGGAGGGTTTGTAGGCCCTAACGCAGCAGGGACCG	G>A	
561	G	+3	Intron	GTGGGAGGGTTTGTAGGCCCTAACGCAGCAGGGACCGGC	C>T	
561	G	-1	Intron	GCCAGGGCTGGTCCCTGAACGCCCTCCGTTCCCTCTGTCCC	G>A/C	
561	H	-1	Intron	GCTACCTCGGGCAGCCCGCGAGCCAGCTCTGTTGTCCAC	G>A	
561	H	-2	Intron	GGCTCCCATTCGAGGACCGCGGGGGCTCACCTGGGGCAGC	G>	
561	H	1	Exon	TCACCCAGCCGCATCTGCCACAGCCACAGGGCACCCCGGT	A>G	
561	J	1	Exon	CTGGAAGATGGGGAAAGGAGCGGCCACAGCGGCACGTCCCA	G>A	
561	M	+1	Intron	AAAATAGTAAGCGCAACCCCTATTTCGACCTTCCCTGTGC	C>A	
561	M	+2	Intron	TATGCCAAAGTCATGAAATGTTGACCCAGTGAATTTCTTG	G>A	
561	M	+3	Intron	GCCAAAGTCATGAAATGTTGACCCAGTGAATTTCTTGGGC	G>A	
561	M	+4	Intron	TTGGGCAAAAGCCACCTACGAACAGGAGTGCACAGTAGTC	G>A	
561	P	+1	Intron	TAAGCAACCTATTAGCCTTTTAACTCTGTCCCGTTCT	T>C	
561	P	1	Exon	GTGTTTAGGGGGAGTGAAAGGGCAGAAAGGCGCTGTGGC	T>C	

561	X	-1	Intron	TCTGTAGGGTAAGGAACACATCTGCTCTGTTACTACTTA	A>T	
561	X	-2	Intron	TCTCTGTGAGGGTAAGGAACACATCTGCTCTGTTACTA	A>C	
561	X	-3	Intron	GACACCCAGATTTTCAGGCAICAAAGTTCTTCTTGCCTCAG	T>A	
561	Y	+1	Intron	ATCTGGGGCCCTGGAGGGAGGGGCTGGCCAGGGAGGAAC	C>G	
561	Y	+2	Intron	TGAGGCACCCAGTGATGCTCTATCCACTATCTGCTGGTTAT	C>T	
561	Y	+3	Intron	CCAGTGATGCTCATCCACTATCTGCTGGTTATCTCTGCTT	A>G	
561	Y	-1	Intron	TACCAAGTCTTAACATGGGGCCACCATCTCACATGTCCT	G>C	
561	Y	-2	Intron	TCCAATTGGCGAGAAGTCCGTTGCTTTTATAGGACACAGA	G>A	
561	Y	-3	Intron	CTCCAATTGGCGAGAAGTCCGTTGCTTTTATAGGACACAG	C>T	
570	C	-1	Intron	TTAACCACTGACCGTATATGGTTTTCATCCTTGAAGACTG	G>C	
570	C	1	Exon	TTAGGTTAAGATCGAGGTCGGGAAGCCACTAGGAGATTTT	C>T	Pro > Leu
570	C	2	Exon	AGCGGCTCTGCTTTTGGGCTCTCTCTGCTGCAAGAGCG	T>C	Val > Ala
570	C	3	Exon	CTTTGTGGTCTCTCTCTGGCAAGAGCGTTTCATCACC	G>A	Gly > Ser
570	C	4	Exon	GAGGCAGTCTTTCACAGACATGTTCAAGATACTGACGTA	C>T	
570	F	+1	Intron	GTTGTGATTGAGATATAGTCTCACACGAGTCGTGCC	T>C	
570	F	-1	Intron	AAGAAATCTTCCAGTTCGGTTGCTCTAACTGAAGAG	C>T	
570	F	1	Exon	ATGTTCTTGTCTATGCTGCTCGGCTTGTGTCAGGTAAGAG	G>A	
570	J	1	Exon	TATTTGAACATTTACTTTTTCTTCTGGCTGCTATTCAAGG	T>C	
581	F	+1	Intron	TGTGGCCACTTTGCTGTTTCAGATTGTCGGTTGGCTTGGCTTGT	G>C	
581	F	+2	Intron	CTTTGCTGTTTCAGATTGTTTCGGTTGGCTTGTATTCTCTG	G>T	
581	F	-1	Intron	TGTACTATTGGCCTCAGGCAATCCACCTCAGCCCCCGAAA	A>G	
698	B	-1	Intron	AGCCTTGCTATTGGCATCAGCTCTTTATTTTTTAAAAAT	C>T	
698	B	1	Exon	CGGGGCCCTGGGGGACACTGCCAGGGCCTGCCATGCTCAT	G>A	Arg > Lys
698	E	1	Exon	AGCCATGGGCATGCAAAATGAGAAAGCAATAATGTAAGTTA	G>A	
698	I	+1	Intron	GTCGCTGCAAGGTTAGTACCTGTGGGGTGGCATTCTA	A>G	
698	I	+2	Intron	GTTATTGATGGGCCAGACTTGGGAAGAACAGACGAGTTG	T>C	
698	I	-1	Intron	TGATGCTGATACGGGATCTCTGTATCCTGCTCTCTCTGTG	T>C	
702	A	-1	Intron	TTTATTAGACACTTTCCGGCAGCTGCCAGGGGAAGAGAC	G>A	
702	B	+1	Intron	ACCTGCTGGAGGTTGGGTGGTGGCCAGGGTGAGGAGCGG	T>C	
702	B	+2	Intron	GGAGTGGCGGCTGGCCAGGGTGAGGAGCGGGTCTCCGT	G>C	
702	B	+3	Intron	GGTGCGTGGCCAGGGTGAGGAGCGGGTCTCCGTGGAGGT	G>C	
702	B	-2	Intron	GTCCAGAGTCAGGGCTCCCAACCCCTTGGGATGCTCGGGAT	A>G	

702	C	1	Exon	GCCCGACAGGCCAGCACCCAGCGAGGTCAGCCGGCCGAGC	G>A	Ala > Thr
702	D	-1	Intron	GGGATGCCTCGATGCCGGCTGCGCCAGAGGGATCTGCAGG	G>A	
702	D	1	Exon	CCTCGTAGGGAGCCCGTAGCGCAGCGGTACCCACCCGGG	C>T	Arg > His
702	F	-1	Intron	GCCCTGTCCTCCGCGCTGCCCAAGGGCCCGCCCTCCACGCCAC	G>	
702	F	1	Exon	GACGCGTGGCCAGATCCGGGTGAAGCTTCTCTTCAA	G>C	Arg > Pro
702	I	1	Exon	TGTGTGGAGACTCACAGGCCGATGGATCTGTGGCTGCGGGC	G>A	Asp > Asn
702	I	3	Exon	CCCAGAGGTGCATGAGCAGAGCTCGTAACCGTCTCCCGAGC	G>A	Val > Ile
722	AA	+2	Intron	CACGCAGTACAGATAATGCCATCTAGTGATACATCTGCCTG	A>G	
722	AA	-1	Intron	GGATGCTTTTAAATGTGGCAATATGAATTAACCATGCATG	A>G	
722	AA	-2	Intron	GCCACCACACCTGGCCAGGTGGTTTATTTAAATGAAGGA	C>T	
722	AA	-3	Intron	CTCAGGTGATCCATCCGCTGGACTCCCAAGTCTGAGA	C>G	
722	AA	-4	Intron	CTGACCTCAGGTGATCCATCCGCTGGACTCCCAAGTGC	C>T	
722	C	1	Exon	GGTGGAGGAGATTAGAAAGATATTGATAAAATAACTCAAT	G>C	Ser>Thr
722	F	+1	Intron	AAGTGAGTAATGGAGACTCCGCTTTGTTAAATCATGTTT	G>A	
722	G	-1	Intron	AAAAATGCTAACAACTATGATGTAGTTGCTAACTATGGT	T>C	
757	A	+1	Intron	ACTTTTGTTAGAGCCCTCCGTAATATACATCTGTGTTT	G>C	
757	A	+2	Intron	GAGTTGCTTAAATAGACTCCGGCCTTACCAATAGTCTCT	C>T	
757	A	+3	Intron	AGGCCAGCCCTCAGAAACCTTCAGTGTACATTTTGTGG	C>T	
757	A	+4	Intron	ACCAAGCCCAATGTTATAGACGTTTGGACTGATTGTGGAA	G>C	
757	A	+5	Intron	GACTGATTTGTGGAAAGGAGGGGGAAGAGGAGAGGATC	G>A	
757	A	+6	Intron	GTCTAGTGTATTCTTTCACAGTCCAGGAAAGAGTGGTTT	A>G	
757	A	-1	Intron	CCGAGCCGGGGCGCTGTGCGCAGCGCTCGGGCCAGGCCGG	G>A	
757	A	2	Exon	TTGCACGAGTTTCGCGCCGCTGGTGGAGTAGGGCTGCCACGG	G>C	
757	A	4	Exon	CTCACCTTCCTCATCGACCCGCGCCGCTTCCGCTACCCCGA	G>C	
757	A	5	Exon	AGCCGGAGAAACCGGCCAGGCTGATCACCACGCGGTGGAT	C>T	

EXAMPLE 10: ALLELE SPECIFIC ASSAY

Once variants were confirmed by sequencing, rapid allele specific assays were designed to type more than 400 individuals (> 200 cases and > 200 controls) for use in the association studies. All coding SNPs (cSNPs) that resulted in an amino acid change were typed. Neutral polymorphisms were typed if: 1) the polymorphism was present in an exon lacking a cSNP; 2) the polymorphism was present in an exon containing a cSNP, but the two polymorphisms were observed to have different frequencies; or 3) the polymorphism was in an intronic region adjacent to an exon without a cSNP. If results from the association studies appeared positive, additional neutral polymorphisms were typed.

Three types of allele specific assays (ASAs) were used. If the SNP resulted in a mutation that created or abolished a restriction site, RFLPs were obtained from PCR products that spanned the variants, and were subsequently analyzed. If the polymorphism did not result in an RFLP, allele-specific oligonucleotide or exonuclease proofreading assays were used. For the allele-specific oligonucleotide assays, PCR products that spanned the polymorphism were electrophoresed on agarose gels and transferred to nylon membranes by Southern blotting. Oligomers 16-20 bp in length were designed such that the middle base was specific for each variant. The oligomers were labeled and successively hybridized to the membrane in order to determine genotypes.

Table 11A, below, shows the information for the ASAs. Column 1 lists the SNP names. Column 2 lists the specific assays used (RFLP or ASO). Column 3 lists the enzymes used in the RFLP assay (described below). Columns 4 and 6 list the sequences of the primers used in the ASO assay (described below). Columns 5 and 7 list the corresponding SEQ ID NOs for the primers. It should be noted that the disclosed SNPs are referred to herein using both short (e.g., 454_E_2; see Table 11A) and long (e.g., Gene 454 E 2; see Examples 11-13) nomenclature.

TABLE 11A: ASA PRIMERS

SNP	ASA Type	RFLP Enzyme	ASO Primer1	SEQ ID NO:	ASO Primer2	SEQ ID NO:	Base change	AA change
214_B_1	RFLP	NdelI					C>T	
214_C_1	ASO		ACCTCAACCCAGGCGTT		CACCTCAACTCAGGCGTTTG		C>T	
214_E_1	RFLP	PvuII					T>C	
214_E_2	RFLP	MspI					G>C	
214_E_3	RFLP	AvaI					C>T	
214_E_4	RFLP		CTCTCTCTGTGAGTGTC		CTCTCTCTTTGAGTGTCCTGG		G>T	Val>Leu
214_E_5	ASO		ACCTCAACCCAGGCGTTT		CCACCTCAACTCAGGCGTTT		C>T	
214_E_6	ASO		TGCTTGACCCCAATCC		GTGCTTGACCTCCTCAATCCG		C>T	Pro>Ser
214_E_7	ASO		TCATACTGCTGCTCTGGC		TATTCACTACTCATGCTCTGGCT		G>A	
214_E_8	ASO		TGTTTAAAGCCACAGCCT		CTGTTTAAAGCCACAGCCTGG		G>A	
214_E_9	ASO		CCCTCGGTTCCACCGTC		GCCATGGCGTGTCTGTC		G>T	Gly>Cys
214_E_10	ASO		GTGCAACTGCTCATCCTG		CGTGCAACTGTTTCATCCTGG		C>T	Leu>Phe
214_E_11	ASO						G>A	
214_E_12	RFLP	DraII					C>T	
214_E_13	RFLP	MwoI					G>A	
214_E_14	RFLP	DraII					C>T	
214_E_15	RFLP	AvaI					C>T	
214_E_16	ASO		GCAGGACACAGTTTCCAGGA		CAGGACACGGTTTCCAG		A>G	Ser>Gly
214_E_17	RFLP	AluNI					C>G	
214_E_18	ASO		CTCAGGAAGGGGCACGCA		CTCAGGAACGGGCACGCA		G>C	
214_E_19	ASO		CTCCCTCCCGCTGCCAC		CTCCCTCTGCCTGCCAC		C>T	
214_E_20	RFLP	XbaI					C>T	
214_E_21	RFLP	HhaI					T>C	
214_E_22	RFLP	Bstul					T>C	Val>Ala
214_E_23	RFLP	PstI					C>T	
214_E_24	RFLP	HpaII					G>A	Gly>Arg

561_Y_+1	RFLP	BsrBI					C>G	
561_Y_-1	RFLP	Fnu4HI					G>C	
570_C_1	RFLP	MspI					C>T	Pro>Leu
570_C_2	ASO		GCCTTTGGCTCTCCTCTG		CTTTGTGGCTTCCCTCT		T>C	Val>Ala
570_C_3	ASO		CTTCACAGACATGTTCAAG		GCCTTCACAGATATGTTCAAGA		G>A	Gly>Ser
570_C_4	RFLP	AluI					C>T	
570_F_1	RFLP	DdeI					G>A	
581_F_+2	ASO		AGATTGTTTCGGTTTGGCTT		TCAGATTGTTCTGTTTGGCTTG		G>T	
598_E_1	ASO		CATGCAATGAGAAAAGCAAT		GGCATGCAATGAAAAAGCAAT		G>A	Arg>Lys
598_I_+1	ASO		CCCACAGGTGACTAACCTT		CCCACAGGCGACTAACC		A>G	
702_A_-1	ASO		ACTTTTCGGCAGCTGC		ACTTTCCGTCAGCTGCC		G>A	
702_B_+1	ASO		AGGTGGGTGTGTGCCAG		GGTGGGTGCGTGGCCA		T>C	
702_B_+3	ASO		AGGGTGAGGAACGGGGT		AGGGTGAGCAACGGGGT		G>C	
702_C_1	RFLP	HaeII					G>A	Ala>Thr
702_D_1	RFLP	HhaI					C>T	Arg>His
702_F_1	RFLP	NciI					G>C	Arg>Pro
702_I_1	RFLP	XbaI					G>A	Asp>Asn
702_I_3	RFLP	DpnII					G>A	Val>Ile
722_C_1	ASO		GATTAGAAACAGTATTGATAA		GATTAGAAACACTATTGATAA		G>C	Ser>Thr
722_F_+1	RFLP	Tth111					G>A	
722_G_-1	ASO		AACAACTATGATTGATTGCTA		CAACTATGACTGTAGTTGC		T>C	
757_A_+4	RFLP	HpyCH4IV					G>C	
757_A_-1	ASO		GCTGTGGCAGCGCTC		CGCTGTGCACAGCGCTCG		G>A	
757_A_2	ASO		GCGCCGCTGGTGGAGTA		GCGCCGCTCGTGGAGTA		G>C	
757_A_4	RFLP	Sau86I					G>C	
757_A_5	RFLP	Cac8I					C>T	

1. RFLP Assay: The amplicon containing the polymorphism was PCR amplified using primers that generated fragments for sequencing (sequencing primers) or SSCP (SSCP primers). The appropriate population of individuals was PCR amplified in 96-well microtiter plates. Enzymes were purchased from NEB. The restriction cocktail containing the appropriate enzyme for the particular polymorphism was added to the PCR product. The reaction was incubated at the appropriate temperature according to the manufacturer's recommendations for 2-3 hr, followed by a 4°C incubation. After digestion, the reactions were size fractionated using the appropriate agarose gel depending on the assay specifications (2.5%, 3%, or Metaphor, FMC Bioproducts). Gels were electrophoresed in 1 X TBE buffer at 170 V for approximately 2 hr. The gel was illuminated using UV, and the image was saved as a Kodak 1D file. Using the Kodak 1D image analysis software, the images were scored and the data was exported to Microsoft® Excel (<http://www.microsoft.com>).
2. ASO assay: The amplicon containing the polymorphism was PCR amplified using primers that generated fragments for sequencing (sequencing primers) or SSCP (SSCP primers). The appropriate population of individuals was PCR amplified in 96-well microtiter plates and re-arrayed into 384-well microtiter plates using a Tecan Genesis RSP200. The amplified products were loaded onto 2% agarose gels and size fractionated at 150V for 5 min. The DNA was transferred from the gel to Hybond N+ nylon membrane (Amersham-Pharmacia) using a Vacuum blotter (Bio-Rad). The filter containing the blotted PCR products was transferred to a dish containing 300 ml pre-hybridization solution (5 X SSPE (pH 7.4), 2% SDS, 5 X Denhardt's). The filter was incubated in pre-hybridization solution at 40°C for over 1 hr. After pre-hybridization, 10 ml of the pre-hybridization solution and the filter were transferred to a washed glass bottle. The allele-specific oligonucleotides (ASO) were designed to contain the polymorphism in the middle of the nucleotide sequence. The size of the oligonucleotide was dependent upon the GC content of the sequence around the polymorphism.

Those ASOs that had a G or C polymorphism were designed so that the T_m was between 54-56°C. Those ASOs that had an A or T polymorphism were designed so that the T_m was between 60-64°C. All oligonucleotides were phosphate-free at the 5' ends and purchased from GibcoBRL. For each polymorphism, 2 ASOs were designed to yield one ASO for each strand.

The ASOs that represented each polymorphism were resuspended at a concentration of 1 µg/µl. Each ASO was end-labeled with γ -ATP³² (6000 Ci/mmol) (NEN) using T4 polynucleotide kinase according to manufacturer recommendations (NEB). The end-labeled products were removed from the unincorporated γ -ATP³² using a Sephadex G-25 column according to the manufacturer's instructions (Amersham-Pharmacia). The entire end-labeled product of one ASO was added to the bottle containing the appropriate filter and 10 ml hybridization solution. The hybridization reaction was placed in a rotisserie oven (Hybaid) and left at 40°C for a minimum of 4 hr. The other ASO was stored at -20°C.

After the prerequisite hybridization time had elapsed, the filter was removed from the bottle and transferred to 1 L of wash solution (0.1 X SSPE (pH 7.4) and 0.1% SDS) pre-warmed to 45°C. After 15 min, the filter was transferred to another liter of wash solution (0.1 X SSPE (pH 7.4) and 0.1% SDS) pre-warmed to 50°C. After 15 min, the filter was wrapped in Saran Wrap®, placed in an autoradiograph cassette, and an X-ray film (Kodak) was placed on top of the filter. Typically, an image was visible within 1 hr. After an image was captured on film following the 50°C wash, images were captured following wash steps at 55°C, 60°C and 65°C. The best image was selected.

The ASO was removed from the filter by adding 1 L of boiling strip solution (0.1 x SSPE (pH 7.4) and 0.1% SDS). This was repeated two more times. After removing the ASO, the filter was pre-hybridized in 300 ml pre-hybridization solution (5 X SSPE (pH 7.4), 2% SDS, and 5 X Denhardt's) at 40°C for over 1 hr. The second end-labeled ASO corresponding to the other strand was removed from storage at -20°C and thawed at RT. The filter was

placed into a glass bottle along with 10 ml hybridization solution and the entire end-labeled product of the second ASO. The hybridization reaction was placed in a rotisserie oven (Hybaid, <http://www.hybaid.co.uk>) and left at 40°C for a minimum of 4 hr. After the hybridization, the filter was washed at various temperatures and images captured on film as described above. The best image for each ASO was converted into a digital image by scanning the film into Adobe® Photoshop®. These images were overlaid using Graphic Converter, and the overlaid images were scored.

3. Exonuclease Proofreading Assay:

Exonuclease Proofreading Assays (EPAs) were also employed (see U.S. Patent No. 5,391,480). Briefly, primers corresponding to the polymorphisms of interest were designed to contain fluorescent tags at the 3' ends. The primers were designed such that the 3' ends contained the variant or consensus nucleotides. Mismatched bases at the 3' ends were removed by an exonuclease proofreading enzyme (Pwo DNA polymerase; Roche, Germany; Cat. No. 1-644-855) in the PCR reaction. Where bases were matched, the resulting PCR products contained the tagged bases. The tagged bases were detected by gel electrophoresis or florescent polarization. Examples of primers used for EPA analysis of Gene 436, Gene 454, Gene 570, and Gene 698 are shown in the Table 11B, below.

TABLE 11B: EPA PRIMERS

SNP	Primer Seq. (5'-3')	SEQ ID NO:
436_K_-2	TTATTCTTTGCGTGCCC	
436_K_-2	ACCTTCCCTTCTCCAAGACC	
436_K_-2	ATTCCAGGCTTCTCAGGAA	
436_K_-2	CGCCTGAGTTTAGCATAGGG	
454_F_-2	CATGGGCTCCCTCGGT	
454_F_-2	CCGGGGAAGTCGATATTGTT	
454_F_-2	CATGGGCTCCCTCGGT	
570_C_2	GCGGTCTTGCTTTTGTGG	
570_C_2	TACTCTGGCGCTCTCCACT	
570_C_2	CGGTCTTGCTTTTGTGG	
698_I_+1	AGAATGGCAACCCACAGG	
698_I_+1	GCTGGTTCTCACGCTGCATATT	
698_I_+1	GTAGAATGGCAACCCACAGG	

EXAMPLE 11: ASSOCIATION STUDY ANALYSIS

1. Case-Control Study: In order to determine whether polymorphisms in candidate genes were associated with the asthma phenotype, association studies were performed using a case-control design. In a well-matched design, the case-control approach is more powerful than the family based transmission disequilibrium test (TDT) (N.E. Morton and A. Collins, 1998, *Proc. Natl. Acad. Sci. USA* 95:11389-93). Case-control studies are, however, sensitive to population admixture.

To avoid issues of population admixture, which can bias case-control studies, unaffected controls were collected in both the US and the UK. A total of three hundred controls were collected, 200 in the UK and 100 in the US. Inclusion into the study required that the control individual was 1) negative for asthma (as determined by self-report of never having asthma); 2) had no first degree relatives with asthma; and 3) was negative for eczema and symptoms indicative of atopy for the past 12 months. Data from an abbreviated questionnaire similar to that administered to the affected sib pair families were collected. Results from skin prick tests to 4 common allergens were also collected. The results of the skin prick tests were used to select a subset of controls that were most likely to be asthma and atopy negative.

A subset of unrelated cases was selected from the affected sib pair families based on the evidence for linkage at the chromosomal location near a given gene. One affected sib demonstrating identity-by-descent (IBD) at the appropriate marker loci was selected from each family. As the appropriate cases may vary for each gene in the region, a larger collection of individuals who were IBD across a larger interval was genotyped. A subset of this collection was used in the analyses. On average, 115 IBD affected individuals and 200 controls were compared for allele and genotype frequencies. This number provided 80% power to detect a difference of 5% or greater between the two groups for a rare allele ($\leq 5\%$) at a 0.05 level of

significance. For a common allele (50%), the number provided 80% power to detect a difference of 10% or more between the two groups.

For each polymorphism, the frequency of the alleles in the control and case populations was compared using a Fisher's exact test. A mutation that increased susceptibility to the disease was expected to be more prevalent in the cases than in the controls, while a protective mutation was expected to be more prevalent in the control group. Similarly, the genotype frequencies of the SNPs were compared between cases and controls. P-values for the allele test were plotted against a coordinate system based on genomic sequence to visualize regions where allelic association was present. A small p-value (or a large value of $-\log(p)$ as plotted in the figures described below) was deemed indicative of an association between the SNPs and the disease phenotype. The analysis was repeated for the US and UK populations, separately, to correct for genetic heterogeneity.

2. Association test with individual SNPs: Chromosomal regions harboring asthma susceptibility genes were identified by association studies using the SNP typing data. Four separate phenotypes were used in these analyses: asthma, bronchial hyper-responsiveness, total IgE, and specific IgE.

a. Asthma Phenotype: A coordinate system was developed based on available genomic sequence, and was used to plot significance values of SNPs and haplotypes according to their relative location along the chromosome (as shown in Figures 11-26). Overlapping genomic sequences were assembled to provide a framework for estimation of relative physical distance between SNPs. Where necessary, gaps were introduced to provide contiguity.

The significance levels (p-values) for allelic association of all typed SNPs to the asthma phenotype are plotted in Figure 11 (combined population) and Figure 12 (US and UK populations, separately). Frequencies and p-values for SNPs associated with the asthma phenotype are shown in Tables 12A, 12B, and 12C for the combined population and for

the UK and US populations, separately. Column 1 lists the SNP names, which were derived from the gene numbers and closest exons. Columns 2 and 3 list the control ("CNTL") allele frequencies and sample sizes ("N"), respectively. Columns 4 and 5 list the affected individuals ("CASE") allele frequencies and sample sizes ("N"), respectively. Columns 6 and 7 list the p-value for the comparison between the case and control allele and genotype frequencies, respectively.

SNPs in Gene 454, Gene 757, Gene 561, and Gene 214 showed a significant association with the asthma phenotype in the combined population, when comparing the allele frequency in the case and control groups (Table 12A). When analyzing the population separately, SNPs in Gene 454, Gene 757, Gene 698 and Gene 561 showed a significant association in the UK population alone (Table 12B), while SNPs in Gene 454 and Gene 561 showed a similar association with the phenotype in the US population (Table 12C). Additional significant results emerged when comparing the genotype frequency of the control and case groups. SNPs in Gene 436 in the combined population, and in Gene 515 and Gene 570 in the US population, also reached statistical significance.

Seven SNPs in Gene 454 showed allelic frequencies significantly different in the cases versus the controls in the combined population. Two SNPs in exon O were more frequent in the controls (19% and 42%, respectively) than in the cases (12% and 33%, respectively). These differences were statistically significant ($p=0.03$ and $p=0.02$), with similar p-values obtained for the genotype comparison ($p=0.03$ and $p=0.01$). The first SNP also reached statistical significance in the UK population ($p=0.03$), while the second SNP had a significant genotype p-value ($p=0.02$) in the UK population. The first SNP in exon O results in an amino acid change of glutamine to arginine. In addition, one SNP in exon M, and one just outside exon M, reached statistical significance in both the US and combined population. These two SNPs showed high linkage disequilibrium and had similar allele frequencies. For the exonic SNP, the p-value was 0.01 for the

combined population, and the allele frequencies were 42% in controls versus 32% in cases. The p-value was 0.02 for the US sample, and the allele frequencies were 41% in controls versus 23% of cases. The genotype comparison was significant ($p=0.02$) in the combined population. The intronic SNP showed significance for both the allele and genotype tests in the combined population ($p=0.007$), for the allele comparison in the US ($p=0.02$), and for the genotype comparison in the UK ($p=0.03$).

Three other SNPs reach statistical significance in the combined population, and in the US or UK populations, alone: 1) SNP E 2, which results in a histidine to tyrosine amino acid change (allele frequencies of 49% in controls and 60% in cases, $p=0.006$ and $p=0.007$ for the allele and genotype test respectively, in the combined population; $p=0.02$ for both allele and genotype tests in the UK population, allele frequencies of 51% in controls and 63% in cases); 2) SNP H 1, an arginine to histidine amino acid change, ($p=0.003$ for the allele and $p=0.002$ for the genotype tests in the combined population, allele frequencies of 22% in the controls and 33% in the cases; $p=0.04$ in the UK population, allele frequencies of 23% in controls and 32% in cases; $p=0.03$ for the allele and $p=0.02$ for the genotype test in the US population, allele frequencies of 20% in controls and 36% in cases); and 3) intronic SNP F -2 ($p=0.008$ for the allele and $p=0.003$ for the genotype tests in the combined population, allele frequencies of 35% in controls and 24% in cases; $p=0.04$ for the allele and $p=0.03$ for the genotype tests in US population, allele frequencies of 37% in cases and 21% in controls).

One SNP in Gene 757 reached statistical significance for the allele test in the combined and UK populations (SNP A 2; $p=0.03$ in the combined population, allele frequencies of 18% in controls and 26% in cases; $p=0.03$ in the UK sample, allele frequencies of 17% in controls and 26% in cases). Another SNP in the same Exon (A 4) reached statistical significance for the genotype test in the combined population ($p<0.05$).

Multiple SNPs in Gene 561 reached statistical significance in either the combined population or in the US or UK populations, separately. SNP J 1 was significant in the combined population ($p=0.04$, allele frequencies of 15% in controls and 9% in cases); SNP Y +1 was significant in the UK sample ($p=0.002$, allele frequencies of 5% in controls and not present in cases); and SNP H 1 was significant in the US population ($p=0.02$, allele frequencies of 10% in controls and 25% in cases). SNP H 1 also showed a significant genotype p -value in the combined population ($p=0.03$) and in the US population ($p=0.01$), while SNP Y +1 showed a significant genotype p -value ($p=0.001$) in the UK population. None of these SNPs resulted in amino acid changes.

A single SNP in Gene 214 reached statistical significance in the combined population ($p=0.04$, allele frequencies of 28% in controls and 36% in cases).

For Gene 436, one SNP (E 1) showed a significant genotype p -value in the combined population ($p=0.04$).

One SNP in Gene 698 (E 1) reached statistical significance in the UK population ($p=0.01$ for the allele test, $p=0.02$ for the genotype test, allele frequencies of 5% in controls and 12% in cases). This SNP results in an arginine to lysine amino acid change.

SNPs in two genes, Gene 515 and Gene 570, showed significant genotype p -values in the US population alone (515 A 1, $p=0.007$; 515 A 2, $p=0.005$; 515 A 4; $p=0.001$; 570 F 1, $p=0.007$).

**TABLE 12A: ASSOCIATION ANALYSIS OF ASTHMA PHENOTYPE
COMBINED US/UK POPULATION**

Combined US and UK						
GENE EXON	FREQUENCIES				ALLELE	GENOTYPE
	CNTL	N	CASE	N	P-VALUE	P-VALUE
454 B 1	7.1%	183	4.1%	98	0.1939	0.1793
454 E -1	25.8%	213	27.0%	113	0.7792	0.9290
454 E 1	0.8%	179	2.7%	112	0.0939	0.2361
454 E 2	49.1%	217	60.3%	117	0.0058	0.0070
454 F -2	34.6%	211	24.3%	115	0.0078	0.0030
454 G -1	8.4%	215	9.7%	113	0.5651	0.4447

454 H 1	22.1%	204	33.0%	112	0.0032	0.0022
454 H 2	2.0%	198	2.6%	115	0.7801	0.7776
454 K 1	1.9%	215	2.6%	114	0.5738	0.5698
454 L -1	6.7%	217	5.5%	109	0.6119	0.9390
454 M 1	42.3%	208	31.6%	98	0.0128	0.0197
454 M 2	6.8%	212	6.3%	111	0.8691	1.0000
454 M +1	43.2%	212	32.3%	113	0.0071	0.0069
454 O 1	19.1%	215	12.2%	107	0.0330	0.0287
454 O 3	17.6%	216	19.2%	112	0.6692	0.1280
454 O 5	3.0%	215	1.9%	106	0.6018	0.5968
454 O 6	42.4%	198	32.9%	111	0.0205	0.0138
436 A 1	1.2%	203	1.9%	106	0.5013	0.3944
436 C -1	15.3%	216	13.6%	114	0.6440	0.6479
436 D 1	5.0%	212	3.5%	114	0.4336	0.7884
436 E 1	46.3%	214	40.6%	112	0.1844	0.0382
436 G 1	10.4%	212	10.6%	109	1.0000	0.5823
436 K -2	14.9%	204	10.4%	111	0.1121	0.1078
436 K +1	4.7%	200	3.6%	112	0.5448	0.8901
436 L -1	4.4%	217	7.8%	115	0.0757	0.0674
436 L 1	1.9%	214	1.8%	113	1.0000	1.0000
515 A 1	43.1%	211	42.0%	106	0.7990	0.2609
515 A 2	37.2%	211	35.2%	105	0.6615	0.2919
515 A 3	7.4%	217	6.6%	113	0.8734	0.8683
515 A 4	43.5%	208	42.1%	108	0.7995	0.1089
515 A 5	4.1%	207	2.3%	108	0.3603	0.3514
515 A 7	2.4%	213	1.4%	110	0.5583	0.5540
570 C 2	9.5%	215	7.7%	91	0.5379	0.8353
570 C 4	9.5%	217	7.2%	90	0.4358	0.7645
570 F 1	47.9%	215	50.6%	89	0.5929	0.5071
757 A 2	18.1%	210	26.3%	99	0.0252	0.0756
757 A 4	1.6%	218	4.6%	98	0.0504	0.0476
757 A +4	39.4%	216	37.0%	104	0.6034	0.4277
698 E 1	6.9%	217	10.9%	105	0.0925	0.1182
698 I +1	32.5%	209	31.9%	102	0.9273	0.8968
561 P 1	34.0%	209	39.4%	104	0.1854	0.4550
561 J 1	14.9%	208	9.0%	105	0.0435	0.1090
561 H 1	9.0%	212	13.7%	102	0.0722	0.0339
561 E 1	0.0%	178	0.6%	87	0.3283	0.3283
561 C 1	0.2%	217	1.0%	104	0.2465	0.2462
561 B +1	13.4%	212	16.1%	90	0.4450	0.6122
561 B 1	48.3%	210	47.2%	90	0.8585	0.9707
561 Y +1	4.7%	212	1.5%	100	0.0658	0.0607
561 X -3	31.3%	214	30.9%	105	1.0000	0.4464
581 F +2	24.3%	216	21.8%	110	0.4956	0.7746
722 C 1	33.0%	209	30.2%	111	0.4779	0.8190
722 F +1	1.4%	217	1.4%	111	1.0000	1.0000
702 A -1	7.0%	215	6.9%	109	1.0000	0.9377
702 C 1	49.3%	213	46.4%	111	0.5081	0.1817
702 D 1	16.1%	217	14.0%	111	0.4948	0.7583

702 F 1	3.7%	217	4.5%	111	0.6734	0.6706
702 I 1	18.6%	204	20.8%	101	0.5159	0.5125
702 I 3	0.7%	217	0.4%	111	1.0000	1.0000
214 B 1	17.8%	214	20.3%	118	0.4666	0.1354
214 E -1	48.8%	202	48.2%	110	0.9332	0.8832
214 E +1	28.3%	217	36.0%	118	0.0445	0.1073

**TABLE 12B: ASSOCIATION ANALYSIS OF ASTHMA PHENOTYPE
UK POPULATION**

UK population						
GENE EXON	FREQUENCIES				ALLELE	GENOTYPE
	CNTL	N	CASE	N	P-VALUE	P-VALUE
454 B 1	7.3%	109	5.0%	70	0.5085	0.4932
454 E -1	25.2%	137	29.8%	84	0.3206	0.5120
454 E 1	1.0%	104	3.7%	82	0.1456	0.2293
454 E 2	51.4%	140	62.6%	87	0.0200	0.0163
454 F -2	33.2%	137	25.3%	87	0.0908	0.1154
454 G -1	8.3%	138	11.3%	84	0.3185	0.3097
454 H 1	23.0%	135	31.9%	83	0.0441	0.0674
454 H 2	2.3%	131	2.3%	86	1.0000	1.0000
454 K 1	2.5%	138	3.0%	84	0.7711	0.7684
454 L -1	5.7%	140	5.0%	80	0.8301	0.8286
454 M 1	42.9%	133	34.7%	72	0.1144	0.1532
454 M 2	5.9%	136	6.1%	82	1.0000	1.0000
454 M +1	44.1%	136	35.1%	84	0.0722	0.0317
454 O 1	19.2%	138	11.0%	77	0.0295	0.0695
454 O 3	16.9%	139	18.3%	82	0.6994	0.3720
454 O 5	2.5%	139	1.9%	78	1.0000	1.0000
454 O 6	44.4%	124	35.4%	82	0.0816	0.0167
436 A 1	1.8%	135	1.9%	78	1.0000	0.7918
436 C -1	13.7%	139	14.3%	84	0.8881	0.8219
436 D 1	3.7%	136	4.2%	84	0.8033	0.7996
436 E 1	45.3%	137	41.0%	83	0.4277	0.0947
436 G 1	9.8%	138	10.0%	80	1.0000	0.8893
436 K -2	13.0%	131	9.8%	82	0.3556	0.5213
436 K +1	2.8%	123	4.3%	82	0.5802	0.5733
436 L -1	4.6%	140	8.2%	85	0.1513	0.1382
436 L 1	1.5%	137	1.8%	83	1.0000	1.0000
515 A 1	45.9%	135	42.2%	77	0.4783	0.7647
515 A 2	39.6%	135	35.5%	76	0.4651	0.7352
515 A 3	7.5%	140	8.4%	83	0.7192	0.7076
515 A 4	45.6%	136	42.8%	83	0.6204	0.7849
515 A 5	3.4%	133	1.8%	81	0.5477	0.5417
515 A 7	1.8%	138	1.8%	82	1.0000	1.0000
570 C 2	8.7%	138	9.6%	68	0.8548	0.9286
570 C 4	8.6%	140	9.0%	67	1.0000	0.9266
570 F 1	49.3%	138	49.3%	67	1.0000	0.7395
757 A 2	17.2%	137	26.0%	75	0.0325	0.0965

757 A 4	1.8%	140	4.7%	74	0.1198	0.1147
757 A +4	40.3%	139	36.9%	80	0.5418	0.3027
698 E 1	5.4%	140	12.3%	81	0.0106	0.0174
698 I +1	38.4%	133	35.3%	78	0.5336	0.8307
561 P 1	33.0%	135	41.3%	80	0.0965	0.2473
561 J 1	13.3%	132	7.4%	81	0.0790	0.1202
561 H 1	8.6%	139	10.6%	80	0.4995	0.2479
561 E 1	0.0%	110	0.0%	68	1.0000	1.0000
561 C 1	0.4%	140	0.6%	80	1.0000	1.0000
561 B +1	15.0%	137	15.9%	66	0.8830	0.8929
561 B 1	52.2%	135	47.0%	66	0.3404	0.6227
561 Y +1	5.5%	136	0.0%	78	0.0016	0.0013
561 X -3	33.0%	138	32.1%	81	0.9160	0.2592
581 F +2	24.6%	140	22.7%	86	0.6515	0.8695
722 C 1	35.0%	133	30.5%	87	0.3523	0.5556
722 F +1	1.8%	139	1.1%	87	0.7121	0.7098
702 A -1	7.2%	138	7.6%	86	1.0000	0.5211
702 C 1	47.8%	136	46.0%	87	0.7706	0.5005
702 D 1	17.9%	140	13.8%	87	0.2963	0.5003
702 F 1	2.9%	140	4.0%	87	0.5914	0.3738
702 I 1	18.3%	131	20.8%	77	0.6065	0.4616
702 I 3	1.1%	140	0.6%	87	1.0000	1.0000
214 B 1	19.2%	138	20.8%	89	0.7181	0.1738
214 E -1	47.9%	140	50.6%	81	0.6218	0.8436
214 E +1	30.7%	140	39.3%	89	0.0686	0.1558

**TABLE 12C: ASSOCIATION ANALYSIS OF ASTHMA PHENOTYPE
US POPULATION**

US population						
GENE EXON	FREQUENCIES			ALLELE P-VALUE	GENOTYPE P-VALUE	
	CNTL	N	CASE			
454 B 1	6.8%	74	1.8%	28	0.2957	0.2815
454 E -1	27.0%	76	19.0%	29	0.2844	0.3531
454 E 1	0.7%	75	0.0%	30	1.0000	1.0000
454 E 2	44.8%	77	53.3%	30	0.2881	0.5554
454 F -2	37.2%	74	21.4%	28	0.0443	0.0288
454 G -1	8.4%	77	5.2%	29	0.5654	0.5481
454 H 1	20.3%	69	36.2%	29	0.0292	0.0199
454 H 2	1.5%	67	3.5%	29	0.5856	0.5818
454 K 1	0.7%	77	1.7%	30	0.4831	0.4840
454 L -1	8.4%	77	6.9%	29	1.0000	0.8698
454 M 1	41.3%	75	23.1%	26	0.0198	0.0792
454 M 2	8.6%	76	6.9%	29	0.7854	1.0000
454 M +1	41.4%	76	24.1%	29	0.0247	0.0815
454 O 1	18.8%	77	15.0%	30	0.5573	0.7174
454 O 3	18.8%	77	21.7%	30	0.7022	0.3960
454 O 5	4.0%	76	1.8%	28	0.6772	0.6713
454 O 6	39.2%	74	25.9%	29	0.0772	0.2334

436 A 1	0.0%	68	1.8%	28	0.2917	0.2917
436 C -1	18.2%	77	11.7%	30	0.3063	0.6276
436 D 1	7.2%	76	1.7%	30	0.1856	0.4802
436 E 1	48.0%	77	39.7%	29	0.2842	0.4465
436 G 1	11.5%	74	12.1%	29	1.0000	0.6979
436 K -2	18.5%	73	12.1%	29	0.3047	0.4804
436 K +1	7.8%	77	1.7%	30	0.1170	0.3281
436 L -1	3.9%	77	6.7%	30	0.4717	0.4616
436 L 1	2.6%	77	1.7%	30	1.0000	1.0000
515 A 1	38.2%	76	41.4%	29	0.7520	0.0067
515 A 2	32.9%	76	34.5%	29	0.8705	0.0048
515 A 3	7.1%	77	1.7%	30	0.1858	0.1724
515 A 4	39.6%	72	40.0%	25	1.0000	0.0010
515 A 5	5.4%	74	3.7%	27	1.0000	1.0000
515 A 7	3.3%	75	0.0%	28	0.3262	0.3197
570 C 2	11.0%	77	2.2%	23	0.0794	0.2617
570 C 4	11.0%	77	2.2%	23	0.0794	0.3161
570 F 1	45.5%	77	54.5%	22	0.3085	0.0071
757 A 2	19.9%	73	27.1%	24	0.3153	0.3078
757 A 4	1.3%	78	4.2%	24	0.2359	0.2347
757 A +4	37.7%	77	37.5%	24	1.0000	1.0000
698 E 1	9.7%	77	6.2%	24	0.5723	1.0000
698 I +1	22.4%	76	20.8%	24	1.0000	0.8691
561 P 1	35.8%	74	33.3%	24	0.8623	0.9473
561 J 1	17.8%	76	14.6%	24	0.8257	1.0000
561 H 1	9.6%	73	25.0%	22	0.0192	0.0123
561 E 1	0.0%	68	2.6%	19	0.2184	0.2184
561 C 1	0.0%	77	2.1%	24	0.2376	0.2376
561 B +1	10.7%	75	16.7%	24	0.3098	0.2553
561 B 1	41.3%	75	47.9%	24	0.5032	0.6426
561 Y +1	3.3%	76	6.8%	22	0.3819	0.3735
561 X -3	28.3%	76	27.1%	24	1.0000	1.0000
581 F +2	23.7%	76	18.8%	24	0.5554	0.8790
722 C 1	29.6%	76	29.2%	24	1.0000	1.0000
722 F +1	0.6%	78	2.1%	24	0.4161	0.4170
702 A -1	6.5%	77	4.3%	23	0.7370	1.0000
702 C 1	52.0%	77	47.9%	24	0.7411	0.5438
702 D 1	13.0%	77	14.6%	24	0.8092	0.6925
702 F 1	5.2%	77	6.2%	24	0.7249	0.7199
702 I 1	19.2%	73	20.8%	24	0.8350	0.9140
702 I 3	0.0%	77	0.0%	24	1.0000	1.0000
214 B 1	15.1%	76	19.0%	29	0.5320	0.4893
214 E -1	50.8%	62	41.4%	29	0.2668	0.4424
214 E +1	24.0%	77	25.9%	29	0.8581	0.7552

b. Bronchial Hyper-responsiveness: The analyses were repeated using asthmatic children with borderline to severe BHR ($PC_{20} \leq 16$)

mg/ml) or PC₂₀(16), as described in the Linkage Analysis section. (Example 3). First, sibling pairs were identified where both sibs were affected and satisfied this new criteria. Of these pairs, one sib was included in the case/control analyses if they showed evidence of linkage at the gene of interest. This phenotype was more restrictive than the Asthma yes/no criteria; hence the number of cases included in the analyses was reduced approximately in half. Where the PC₂₀(16) subgroup represented a more genetically homogeneous sample, one could expect an increase in the effect size compared to the one observed in the original set of cases. However, the reduction in sample size could result in estimates that were less accurate. This, in turn, could obscure a trend in allele frequencies in the control group, the original set of cases, and the PC₂₀(16) subgroup. In addition, the reduction in sample size could induce a reduction in power (and increase in p-values) in spite of the larger effect size.

The significance levels (p-values) for allelic association of all typed SNPs to the BHR phenotype are plotted in Figure 13 (combined population) and Figure 14 (US and UK populations, separately). Frequencies and p-values for SNPs associated with the BHR phenotype are shown in Tables 13A, 13B, and 13C for the combined population and for the UK and US populations separately.

**TABLE 13A: ASSOCIATION ANALYSIS OF BHR PHENOTYPE
COMBINED US/UK POPULATION**

Combined US and UK						
GENE EXON	FREQUENCIES				ALLELE P-VALUE	GENOTYPE P-VALUE
	CNTL	N	CASE	N		
454 B 1	7.1%	183	4.3%	46	0.4798	0.4636
454 E -1	25.8%	213	29.8%	52	0.4577	0.5705
454 E 1	0.8%	179	2.9%	51	0.1260	0.1246
454 E 2	49.1%	217	63.6%	55	0.0074	0.0140
454 F -2	34.6%	211	24.6%	55	0.0519	0.0312
454 G -1	8.4%	215	12.5%	52	0.1890	0.1353
454 H 1	22.1%	204	33.0%	53	0.0223	0.0181
454 H 2	2.0%	198	3.7%	54	0.2962	0.2923
454 K 1	1.9%	215	3.8%	53	0.2664	0.2621
454 L -1	6.7%	217	6.0%	50	1.0000	0.8944
454 M 1	42.3%	208	35.2%	44	0.2349	0.4893

454 M 2	6.8%	212	6.9%	51	1.0000	0.8043
454 M +1	43.2%	212	35.9%	53	0.1873	0.3681
454 O 1	19.1%	215	12.8%	47	0.1816	0.3182
454 O 3	17.6%	216	17.6%	51	1.0000	0.2734
454 O 5	3.0%	215	2.1%	48	1.0000	1.0000
454 O 6	42.4%	198	36.3%	51	0.3100	0.5059
436 A 1	1.2%	203	2.0%	49	0.6263	0.3975
436 C -1	15.3%	216	12.3%	53	0.5402	0.7508
436 D 1	5.0%	212	0.9%	53	0.0971	0.1825
436 E 1	46.3%	214	45.1%	51	0.9120	0.2363
436 G 1	10.4%	212	11.0%	50	0.8565	0.4689
436 K -2	14.9%	204	12.3%	53	0.5378	0.5523
436 K +1	4.7%	200	1.0%	52	0.0925	0.3121
436 L -1	4.4%	217	6.5%	54	0.3256	0.4368
436 L 1	1.9%	214	1.9%	53	1.0000	1.0000
515 A 1	43.1%	211	43.5%	46	1.0000	1.0000
515 A 2	37.2%	211	33.3%	45	0.5472	0.7363
515 A 3	7.4%	217	4.7%	53	0.3976	0.3791
515 A 4	43.5%	208	42.9%	49	1.0000	0.8566
515 A 5	4.1%	207	3.1%	48	1.0000	0.7745
515 A 7	2.4%	213	2.0%	51	1.0000	1.0000
570 C 2	9.5%	215	9.5%	42	1.0000	0.7549
570 C 4	9.5%	217	9.8%	41	1.0000	0.9219
570 F 1	47.9%	215	51.2%	41	0.6303	0.7776
757 A 2	18.1%	210	29.1%	43	0.0260	0.0659
757 A 4	1.6%	218	4.8%	42	0.0849	0.0826
757 A +4	39.4%	216	38.9%	45	1.0000	0.7300
698 E 1	6.9%	217	9.8%	46	0.3791	0.4289
698 I +1	32.5%	209	29.1%	43	0.6118	0.4244
561 P 1	34.0%	209	31.5%	46	0.7151	0.9229
561 J 1	14.9%	208	7.6%	46	0.0661	0.2215
561 H 1	9.0%	212	11.1%	45	0.5495	0.2181
561 E 1	0.0%	178	1.3%	38	0.1759	0.1759
561 C 1	0.2%	217	0.0%	46	1.0000	1.0000
561 B +1	13.4%	212	14.5%	38	0.8557	0.2404
561 B 1	48.3%	210	47.4%	38	0.9013	0.6065
561 Y +1	4.7%	212	0.0%	45	0.0329	0.0296
561 X -3	31.3%	214	32.6%	46	0.8057	0.2221
581 F +2	24.3%	216	16.0%	50	0.0852	0.1792
722 C 1	33.0%	209	27.0%	50	0.2827	0.1568
722 F +1	1.4%	217	1.0%	50	1.0000	1.0000
702 A -1	7.0%	215	8.2%	49	0.6666	0.7105
702 C 1	49.3%	213	42.0%	50	0.2212	0.0884
702 D 1	16.1%	217	11.0%	50	0.2193	0.5230
702 F 1	3.7%	217	5.0%	50	0.5674	0.4866
702 I 1	18.6%	204	19.1%	47	0.8842	0.0607
702 I 3	0.7%	217	1.0%	50	0.5648	0.5660
214 B 1	17.8%	214	24.0%	52	0.1630	0.0098
214 E -1	48.8%	202	44.9%	49	0.5014	0.4228

214 E +1	28.3%	217	38.5%	52	0.0568	0.0629
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**TABLE 13B: ASSOCIATION ANALYSIS OF BHR PHENOTYPE
UK POPULATION**

UK population						
GENE EXON	FREQUENCIES				ALLELE P-VALUE	GENOTYPE P-VALUE
	CNTL	N	CASE	N		
454 B 1	7.3%	109	5.6%	36	0.7903	0.7821
454 E -1	25.2%	137	30.9%	42	0.3234	0.2916
454 E 1	1.0%	104	3.8%	40	0.1331	0.1311
454 E 2	51.4%	140	65.9%	44	0.0196	0.0428
454 F -2	33.2%	137	25.0%	44	0.1858	0.2137
454 G -1	8.3%	138	13.1%	42	0.2033	0.1620
454 H 1	23.0%	135	33.3%	42	0.0629	0.0498
454 H 2	2.3%	131	4.7%	43	0.2708	0.2656
454 K 1	2.5%	138	3.6%	42	0.7041	0.7005
454 L -1	5.7%	140	5.0%	40	1.0000	1.0000
454 M 1	42.9%	133	37.5%	36	0.5008	0.7446
454 M 2	5.9%	136	6.1%	41	1.0000	0.8275
454 M +1	44.1%	136	38.1%	42	0.3776	0.5479
454 O 1	19.2%	138	11.1%	36	0.1197	0.3349
454 O 3	16.9%	139	15.0%	40	0.7360	0.6730
454 O 5	2.5%	139	1.3%	38	1.0000	1.0000
454 O 6	44.4%	124	37.8%	41	0.3068	0.4297
436 A 1	1.8%	135	2.6%	38	0.6510	0.4579
436 C -1	13.7%	139	11.9%	42	0.8544	1.0000
436 D 1	3.7%	136	1.2%	42	0.4697	0.4625
436 E 1	45.3%	137	46.3%	41	0.8998	0.2517
436 G 1	9.8%	138	10.3%	39	0.8334	0.5314
436 K -2	13.0%	131	11.9%	42	1.0000	1.0000
436 K +1	2.8%	123	1.2%	41	0.6847	0.6807
436 L -1	4.6%	140	8.1%	43	0.2747	0.2611
436 L 1	1.5%	137	2.4%	42	0.6283	0.6262
515 A 1	45.9%	135	43.1%	36	0.6912	0.5660
515 A 2	39.6%	135	32.9%	35	0.3355	0.5485
515 A 3	7.5%	140	5.9%	42	0.8101	0.8024
515 A 4	45.6%	136	43.9%	41	0.8016	0.9215
515 A 5	3.4%	133	2.5%	40	1.0000	1.0000
515 A 7	1.8%	138	2.4%	41	0.6619	0.6604
570 C 2	8.7%	138	10.3%	34	0.6413	0.5472
570 C 4	8.6%	140	10.6%	33	0.6321	0.4646
570 F 1	49.3%	138	48.5%	33	1.0000	0.1921
757 A 2	17.2%	137	27.1%	35	0.0632	0.1182
757 A 4	1.8%	140	4.4%	34	0.1910	0.1890
757 A +4	40.3%	139	40.5%	37	1.0000	0.7080
698 E 1	5.4%	140	10.5%	38	0.1161	0.1501
698 I +1	38.4%	133	32.9%	35	0.4873	0.4925
561 P 1	33.0%	135	34.2%	38	0.8906	1.0000

561 J 1	13.3%	132	5.3%	38	0.0650	0.1740
561 H 1	8.6%	139	8.1%	37	1.0000	1.0000
561 E 1	0.0%	110	0.0%	32	1.0000	1.0000
561 C 1	0.4%	140	0.0%	38	1.0000	1.0000
561 B +1	15.0%	137	13.3%	30	0.8425	0.6278
561 B 1	52.2%	135	45.0%	30	0.3217	0.3440
561 Y +1	5.5%	136	0.0%	38	0.0486	0.0437
561 X -3	33.0%	138	35.5%	38	0.6825	0.0827
581 F +2	24.6%	140	18.6%	43	0.3068	0.3581
722 C 1	35.0%	133	27.9%	43	0.2393	0.2426
722 F +1	1.8%	139	1.2%	43	1.0000	1.0000
702 A -1	7.2%	138	8.3%	42	0.8130	0.8054
702 C 1	47.8%	136	41.9%	43	0.3856	0.3789
702 D 1	17.9%	140	10.5%	43	0.1308	0.2813
702 F 1	2.9%	140	4.7%	43	0.4871	0.4261
702 I 1	18.3%	131	18.8%	40	1.0000	0.1367
702 I 3	1.1%	140	1.2%	43	1.0000	1.0000
214 B 1	19.2%	138	25.6%	43	0.2235	0.0237
214 E -1	47.9%	140	45.0%	40	0.7039	0.6187
214 E +1	30.7%	140	40.7%	43	0.0900	0.1646

**TABLE 13C: ASSOCIATION ANALYSIS OF BHR PHENOTYPE
US POPULATION**

US population						
GENE EXON	FREQUENCIES				ALLELE	GENOTYPE
	CNTL	N	CASE	N	P-VALUE	P-VALUE
454 B 1	6.8%	74	0.0%	10	0.6099	0.5997
454 E -1	27.0%	76	25.0%	10	1.0000	0.8773
454 E 1	0.7%	75	0.0%	11	1.0000	1.0000
454 E 2	44.8%	77	54.5%	11	0.4939	0.6511
454 F -2	37.2%	74	22.7%	11	0.2356	0.3215
454 G -1	8.4%	77	10.0%	10	0.6842	0.6809
454 H 1	20.3%	69	31.8%	11	0.2665	0.2593
454 H 2	1.5%	67	0.0%	11	1.0000	1.0000
454 K 1	0.7%	77	4.5%	11	0.2350	0.2356
454 L -1	8.4%	77	10.0%	10	0.6842	0.6961
454 M 1	41.3%	75	25.0%	8	0.2845	0.5385
454 M 2	8.6%	76	10.0%	10	0.6875	0.6984
454 M +1	41.4%	76	27.3%	11	0.2483	0.5213
454 O 1	18.8%	77	18.2%	11	1.0000	0.8406
454 O 3	18.8%	77	27.3%	11	0.3924	0.2974
454 O 5	4.0%	76	5.0%	10	0.5860	1.0000
454 O 6	39.2%	74	30.0%	10	0.4733	0.8144
436 A 1	0.0%	68	0.0%	11	1.0000	1.0000
436 C -1	18.2%	77	13.6%	11	0.7696	1.0000
436 D 1	7.2%	76	0.0%	11	0.3630	0.6475
436 E 1	48.0%	77	40.0%	10	0.6353	0.8165

436 G 1	11.5%	74	13.6%	11	0.7268	0.5735
436 K -2	18.5%	73	13.6%	11	0.7686	1.0000
436 K +1	7.8%	77	0.0%	11	0.3665	0.4304
436 L -1	3.9%	77	0.0%	11	1.0000	1.0000
436 L 1	2.6%	77	0.0%	11	1.0000	1.0000
515 A 1	38.2%	76	45.0%	10	0.6283	0.2911
515 A 2	32.9%	76	35.0%	10	1.0000	0.1469
515 A 3	7.1%	77	0.0%	11	0.3634	0.3458
515 A 4	39.6%	72	37.5%	8	1.0000	0.1886
515 A 5	5.4%	74	6.2%	8	1.0000	1.0000
515 A 7	3.3%	75	0.0%	10	1.0000	1.0000
570 C 2	11.0%	77	6.2%	8	1.0000	1.0000
570 C 4	11.0%	77	6.2%	8	1.0000	1.0000
570 F 1	45.5%	77	62.5%	8	0.2925	0.0453
757 A 2	19.9%	73	37.5%	8	0.1158	0.2069
757 A 4	1.3%	78	6.2%	8	0.2553	0.2566
757 A +4	37.7%	77	31.3%	8	0.7873	1.0000
698 E 1	9.7%	77	6.2%	8	1.0000	1.0000
698 I +1	22.4%	76	12.5%	8	0.5269	1.0000
561 P 1	35.8%	74	18.8%	8	0.2668	0.6666
561 J 1	17.8%	76	18.8%	8	1.0000	0.7579
561 H 1	9.6%	73	25.0%	8	0.0826	0.0677
561 E 1	0.0%	68	8.3%	6	0.0811	0.0811
561 C 1	0.0%	77	0.0%	8	1.0000	1.0000
561 B +1	10.7%	75	18.8%	8	0.3996	0.1039
561 B 1	41.3%	75	56.3%	8	0.2937	0.4467
561 Y +1	3.3%	76	0.0%	7	1.0000	1.0000
561 X -3	28.3%	76	18.8%	8	0.5610	1.0000
581 F +2	23.7%	76	0.0%	7	0.0416	0.2351
722 C 1	29.6%	76	21.4%	7	0.7592	1.0000
722 F +1	0.6%	78	0.0%	7	1.0000	1.0000
702 A -1	6.5%	77	7.1%	7	1.0000	0.6027
702 C 1	52.0%	77	42.9%	7	0.5841	0.1734
702 D 1	13.0%	77	14.3%	7	1.0000	0.7000
702 F 1	5.2%	77	7.1%	7	0.5522	0.5618
702 I 1	19.2%	73	21.4%	7	0.7357	0.3487
702 I 3	0.0%	77	0.0%	7	1.0000	1.0000
214 B 1	15.1%	76	16.7%	9	0.7414	1.0000
214 E -1	50.8%	62	44.4%	9	0.8016	0.7277
214 E +1	24.0%	77	27.8%	9	0.7732	0.4601

The results for the BHR sub-phenotype closely resembled the results observed for the asthma phenotype, described above. Namely, SNPs in Gene 454, Gene 757, and Gene 561 showed a significant association with the BHR phenotype in the combined population when comparing allele frequencies in the control and case populations. When analyzing the

populations separately, SNPs in Gene 454 and Gene 561 showed a significant association in the UK population alone, while SNPs in Gene 581 showed a similar association with the phenotype in the US population. In addition, the genotypic comparison yielded significant results for SNPs in
 5 Gene 570 in the US population and in Gene 214, Gene 454 and Gene 561 for the UK and combined population (see Tables 13A-13C).

The most significant results were obtained for Gene 454, where SNP E 2 showed a p-value of 0.007 for the allele test and p-value of 0.01 for the genotype test in the combined population (49% in control vs. 64% in cases).
 10 SNP E 2 was also significant in the UK population alone for the allele (p=0.02) and genotype (p=0.04) tests. Two more SNPs reached statistical significance in Gene 454 for this sub-phenotype: 1) SNP H 1 (p=0.02 in the combined population, 22% in controls vs. 33% in cases; p<0.05 for genotypic test in the UK population); and 2) SNP F -2 (genotypic p-value of
 15 0.03 in the combined population).

For Gene 757, SNP A 2 was significant with a p-value of 0.03 in the combined population (18% in controls vs. 29% in cases).

One SNP in Gene 561 was significant in both the combined population and in the UK population alone (p=0.03 for both the allele and
 20 genotype tests in the combined population, 5% in controls vs. not present in cases; p<0.05 for allele and p=0.04 for genotype in UK, 6% in controls vs. not present in cases).

Gene 214 was significant in both the combined and UK populations when comparing the genotype frequencies between the cases and controls
 25 (p=0.01 combined population, p=0.02 UK population).

In the US population, one SNP in Gene 581 reached statistical significance (F +2, p=0.04, 24% in controls vs. not present in cases). The comparison of genotype frequencies also yielded a significant result for Gene 570 (SNP F 1, p<0.05).

30 c. Total IgE: The analyses were performed using asthmatic children with elevated total IgE levels, as described in the Linkage

- Analysis section (Example 3). First, sibling pairs were identified where both sibs were affected and satisfied this new criteria. Of these pairs, one sib was included in the case/control analyses if they showed evidence of linkage at the gene of interest. This phenotype was more restrictive than the
- 5 Asthma yes/no criteria; hence the number of cases included in the analyses was reduced by approximately 41%.

- The significance levels (p-values) for allelic association of all typed SNPs to the IgE phenotype are plotted in Figure 15 (combined population) and Figure 16 (US and UK populations, separately). Frequencies and p-
- 10 values for SNPs associated with the IgE phenotype are shown in Tables 14A, 14B, and 14C for the combined population and for the UK and US populations, separately.

**TABLE 14A: ASSOCIATION ANALYSIS OF TOTAL IgE PHENOTYPE
COMBINED US/UK POPULATION**

Combined US and UK						
GENE EXON	FREQUENCIES				ALLELE P-VALUE	GENOTYPE P-VALUE
	CNTL	N	CASE	N		
454 B 1	7.1%	183	5.2%	58	0.5295	0.5138
454 E -1	25.8%	213	24.6%	69	0.8230	0.9474
454 E 1	0.8%	179	2.2%	67	0.3519	0.3491
454 E 2	49.1%	217	58.3%	72	0.0552	0.0364
454 F -2	34.6%	211	22.5%	71	0.0089	0.0146
454 G -1	8.4%	215	10.1%	69	0.4950	0.3130
454 H 1	22.1%	204	35.3%	68	0.0030	0.0010
454 H 2	2.0%	198	4.2%	71	0.2146	0.2088
454 K 1	1.9%	215	2.9%	69	0.4976	0.4937
454 L -1	6.7%	217	6.0%	67	1.0000	0.9236
454 M 1	42.3%	208	29.3%	58	0.0133	0.0399
454 M 2	6.8%	212	6.6%	68	1.0000	1.0000
454 M +1	43.2%	212	31.2%	69	0.0127	0.0283
454 O 1	19.1%	215	8.6%	64	0.0044	0.0211
454 O 3	17.6%	216	18.4%	68	0.8977	0.1305
454 O 5	3.0%	215	1.6%	63	0.5387	0.5327
454 O 6	42.4%	198	31.3%	67	0.0248	0.0468
436 A 1	1.2%	203	1.6%	64	0.6751	0.6934
436 C -1	15.3%	216	13.8%	69	0.7838	1.0000
436 D 1	5.0%	212	3.6%	69	0.6444	0.8539
436 E 1	46.3%	214	39.0%	68	0.1390	0.1920
436 G 1	10.4%	212	10.0%	65	1.0000	0.9441
436 K -2	14.9%	204	7.5%	67	0.0266	0.1029

436 K +1	4.7%	200	3.6%	69	0.8111	1.0000
436 L -1	4.4%	217	5.7%	70	0.4967	0.4872
436 L 1	1.9%	214	1.5%	69	1.0000	1.0000
515 A 1	43.1%	211	43.6%	63	0.9188	0.5757
515 A 2	37.2%	211	37.1%	62	1.0000	0.6857
515 A 3	7.4%	217	7.2%	69	1.0000	1.0000
515 A 4	43.5%	208	43.9%	66	1.0000	0.3943
515 A 5	4.1%	207	1.5%	66	0.2720	0.1767
515 A 7	2.4%	213	1.5%	67	0.7400	0.7370
570 C 2	9.5%	215	9.8%	51	1.0000	0.9293
570 C 4	9.5%	217	9.2%	49	1.0000	1.0000
570 F 1	47.9%	215	49.0%	50	0.9116	0.9785
757 A 2	18.1%	210	25.9%	54	0.0778	0.1407
757 A 4	1.6%	218	4.5%	55	0.0723	0.0700
757 A +4	39.4%	216	41.2%	57	0.7473	0.0453
698 E 1	6.9%	217	11.2%	58	0.1706	0.2526
698 I +1	32.5%	209	34.5%	55	0.7326	0.9308
561 P 1	34.0%	209	40.4%	57	0.2246	0.1509
561 J 1	14.9%	208	10.3%	58	0.2286	0.4828
561 H 1	9.0%	212	9.1%	55	1.0000	1.0000
561 E 1	0.0%	178	1.0%	48	0.2124	0.2124
561 C 1	0.2%	217	0.9%	57	0.3731	0.3734
561 B +1	13.4%	212	14.3%	49	0.8701	0.8764
561 B 1	48.3%	210	44.9%	49	0.5754	0.7216
561 Y +1	4.7%	212	1.8%	55	0.2788	0.2683
561 X -3	31.3%	214	30.2%	58	0.9100	0.9259
581 F +2	24.3%	216	22.1%	61	0.7182	0.7766
722 C 1	33.0%	209	30.3%	61	0.6602	0.7945
722 F +1	1.4%	217	0.8%	61	1.0000	1.0000
702 A -1	7.0%	215	7.5%	60	0.8413	0.5612
702 C 1	49.3%	213	45.1%	61	0.4721	0.3089
702 D 1	16.1%	217	13.9%	61	0.6723	0.7607
702 F 1	3.7%	217	6.6%	61	0.2044	0.2132
702 I 1	18.6%	204	22.3%	56	0.4185	0.3178
702 I 3	0.7%	217	0.8%	61	1.0000	1.0000
214 B 1	17.8%	214	20.0%	65	0.6044	0.0517
214 E -1	48.8%	202	50.0%	59	0.8347	0.5796
214 E +1	28.3%	217	38.5%	65	0.0305	0.0637

**TABLE 14B: ASSOCIATION ANALYSIS OF TOTAL IgE PHENOTYPE
UK POPULATION**

UK population						
GENE EXON	FREQUENCIES				ALLELE P-VALUE	GENOTYPE P-VALUE
	CNTL	N	CASE	N		
454 B 1	7.3%	109	5.3%	47	0.6272	0.6139
454 E -1	25.2%	137	27.6%	58	0.6154	0.8376
454 E 1	1.0%	104	2.7%	56	0.3478	0.3443
454 E 2	51.4%	140	59.8%	61	0.1286	0.1334

454 F -2	33.2%	137	24.6%	61	0.0983	0.1632
454 G -1	8.3%	138	11.2%	58	0.4433	0.3051
454 H 1	23.0%	135	29.8%	57	0.1586	0.1000
454 H 2	2.3%	131	3.3%	60	0.5130	0.5089
454 K 1	2.5%	138	3.5%	58	0.7384	0.7348
454 L -1	5.7%	140	6.2%	56	0.8151	0.7942
454 M 1	42.9%	133	33.7%	49	0.1192	0.2262
454 M 2	5.9%	136	7.0%	57	0.6502	0.8070
454 M +1	44.1%	136	34.5%	58	0.0911	0.0729
454 O 1	19.2%	138	8.5%	53	0.0125	0.0413
454 O 3	16.9%	139	18.4%	57	0.7693	0.1485
454 O 5	2.5%	139	1.9%	53	1.0000	1.0000
454 O 6	44.4%	124	34.8%	56	0.1056	0.0462
436 A 1	1.8%	135	1.8%	54	1.0000	0.7330
436 C -1	13.7%	139	14.7%	58	0.8734	0.8810
436 D 1	3.7%	136	4.3%	58	0.7771	0.7732
436 E 1	45.3%	137	40.4%	57	0.4319	0.1736
436 G 1	9.8%	138	10.0%	55	1.0000	0.9280
436 K -2	13.0%	131	7.0%	57	0.1094	0.2734
436 K +1	2.8%	123	4.3%	58	0.5325	0.5259
436 L -1	4.6%	140	5.9%	59	0.6184	0.6095
436 L 1	1.5%	137	0.9%	58	1.0000	1.0000
515 A 1	45.9%	135	43.3%	52	0.7281	0.9116
515 A 2	39.6%	135	36.3%	51	0.6334	0.8401
515 A 3	7.5%	140	8.6%	58	0.6854	0.6732
515 A 4	45.6%	136	43.9%	57	0.8227	0.9364
515 A 5	3.4%	133	1.7%	57	0.5167	0.5100
515 A 7	1.8%	138	1.8%	56	1.0000	1.0000
570 C 2	8.7%	138	10.6%	47	0.5421	0.5764
570 C 4	8.6%	140	10.0%	45	0.6735	0.5645
570 F 1	49.3%	138	48.9%	46	1.0000	0.6579
757 A 2	17.2%	137	26.7%	45	0.0649	0.0752
757 A 4	1.8%	140	4.3%	46	0.2332	0.2281
757 A +4	40.3%	139	40.6%	48	1.0000	0.0252
698 E 1	5.4%	140	13.3%	49	0.0140	0.0180
698 I +1	38.4%	133	35.9%	46	0.7094	0.6131
561 P 1	33.0%	135	40.6%	48	0.2126	0.2794
561 J 1	13.3%	132	7.1%	49	0.1388	0.3083
561 H 1	8.6%	139	6.2%	48	0.5218	0.5022
561 E 1	0.0%	110	0.0%	41	1.0000	1.0000
561 C 1	0.4%	140	0.0%	48	1.0000	1.0000
561 B +1	15.0%	137	15.0%	40	1.0000	1.0000
561 B 1	52.2%	135	46.3%	40	0.3744	0.5410
561 Y +1	5.5%	136	0.0%	47	0.0148	0.0129
561 X -3	33.0%	138	32.6%	49	1.0000	0.7487
581 F +2	24.6%	140	21.7%	53	0.5941	0.8802
722 C 1	35.0%	133	29.3%	53	0.3304	0.4590
722 F +1	1.8%	139	0.9%	53	1.0000	1.0000
702 A -1	7.2%	138	7.7%	52	0.8294	0.2921

702 C 1	47.8%	136	45.3%	53	0.7309	0.7299
702 D 1	17.9%	140	14.1%	53	0.4476	0.6600
702 F 1	2.9%	140	5.7%	53	0.2227	0.1546
702 I 1	18.3%	131	20.8%	48	0.6485	0.4405
702 I 3	1.1%	140	0.9%	53	1.0000	1.0000
214 B 1	19.2%	138	21.3%	54	0.6700	0.1252
214 E -1	47.9%	140	52.1%	48	0.4807	0.5675
214 E +1	30.7%	140	38.9%	54	0.1482	0.2246

**TABLE 14C: ASSOCIATION ANALYSIS OF TOTAL IgE PHENOTYPE
US POPULATION**

US population						
GENE EXON	FREQUENCIES				ALLELE P-VALUE	GENOTYPE P-VALUE
	CNTL	N	CASE	N		
454 B 1	6.8%	74	4.5%	11	1.0000	1.0000
454 E -1	27.0%	76	9.1%	11	0.1094	0.3156
454 E 1	0.7%	75	0.0%	11	1.0000	1.0000
454 E 2	44.8%	77	50.0%	11	0.6553	0.5025
454 F -2	37.2%	74	10.0%	10	0.0214	0.1140
454 G -1	8.4%	77	4.5%	11	1.0000	1.0000
454 H 1	20.3%	69	63.6%	11	0.0001	0.0001
454 H 2	1.5%	67	9.1%	11	0.0957	0.0932
454 K 1	0.7%	77	0.0%	11	1.0000	1.0000
454 L -1	8.4%	77	4.5%	11	1.0000	1.0000
454 M 1	41.3%	75	5.6%	9	0.0034	0.0204
454 M 2	8.6%	76	4.5%	11	1.0000	1.0000
454 M +1	41.4%	76	13.6%	11	0.0170	0.0132
454 O 1	18.8%	77	9.1%	11	0.3747	0.8371
454 O 3	18.8%	77	18.2%	11	1.0000	0.8248
454 O 5	4.0%	76	0.0%	10	1.0000	1.0000
454 O 6	39.2%	74	13.6%	11	0.0299	0.0138
436 A 1	0.0%	68	0.0%	10	1.0000	1.0000
436 C -1	18.2%	77	9.1%	11	0.3766	0.8138
436 D 1	7.2%	76	0.0%	11	0.3630	0.6475
436 E 1	48.0%	77	31.8%	11	0.1757	0.1728
436 G 1	11.5%	74	10.0%	10	1.0000	1.0000
436 K -2	18.5%	73	10.0%	10	0.5324	1.0000
436 K +1	7.8%	77	0.0%	11	0.3665	0.4304
436 L -1	3.9%	77	4.5%	11	1.0000	1.0000
436 L 1	2.6%	77	4.5%	11	0.4913	0.4957
515 A 1	38.2%	76	45.5%	11	0.6409	0.0155
515 A 2	32.9%	76	40.9%	11	0.4765	0.0234
515 A 3	7.1%	77	0.0%	11	0.3634	0.3458
515 A 4	39.6%	72	44.4%	9	0.7999	0.0332
515 A 5	5.4%	74	0.0%	9	0.6008	0.5894
515 A 7	3.3%	75	0.0%	11	1.0000	1.0000
570 C 2	11.0%	77	0.0%	4	1.0000	1.0000

570 C 4	11.0%	77	0.0%	4	1.0000	1.0000
570 F 1	45.5%	77	50.0%	4	1.0000	0.0375
757 A 2	19.9%	73	22.2%	9	0.7616	0.6620
757 A 4	1.3%	78	5.6%	9	0.2808	0.2823
757 A +4	37.7%	77	44.4%	9	0.6140	0.7660
698 E 1	9.7%	77	0.0%	9	0.3721	0.6785
698 I +1	22.4%	76	27.8%	9	0.5652	0.3464
561 P 1	35.8%	74	38.9%	9	0.7997	0.2279
561 J 1	17.8%	76	27.8%	9	0.3388	0.3272
561 H 1	9.6%	73	28.6%	7	0.0550	0.0419
561 E 1	0.0%	68	7.1%	7	0.0933	0.0933
561 C 1	0.0%	77	5.6%	9	0.1047	0.1047
561 B +1	10.7%	75	11.1%	9	1.0000	1.0000
561 B 1	41.3%	75	38.9%	9	1.0000	1.0000
561 Y +1	3.3%	76	12.5%	8	0.1348	0.1312
561 X -3	28.3%	76	16.7%	9	0.4045	0.7647
581 F +2	23.7%	76	25.0%	8	1.0000	0.1246
722 C 1	29.6%	76	37.5%	8	0.5706	0.6624
722 F +1	0.6%	78	0.0%	8	1.0000	1.0000
702 A -1	6.5%	77	6.2%	8	1.0000	1.0000
702 C 1	52.0%	77	43.8%	8	0.6050	0.4101
702 D 1	13.0%	77	12.5%	8	1.0000	1.0000
702 F 1	5.2%	77	12.5%	8	0.2393	0.2370
702 I 1	19.2%	73	31.3%	8	0.3233	0.3168
702 I 3	0.0%	77	0.0%	8	1.0000	1.0000
214 B 1	15.1%	76	13.6%	11	1.0000	1.0000
214 E -1	50.8%	62	40.9%	11	0.4894	0.5711
214 E +1	24.0%	77	36.4%	11	0.2943	0.2456

For the total IgE phenotype, SNPs in Gene 454, Gene 436 and Gene 214 showed a significant association in the combined population when comparing the allele frequencies in the case and control groups. When analyzing the population separately, SNPs in gene 454 were significant in both the UK and US populations, separately, while SNPs in Gene 698 and Gene 561 showed a significant association in the UK population. Additional significant results were identified when comparing the genotype frequencies in the case and control groups. SNPs in Gene 454 (US, UK, and combined), Gene 515 (US), Gene 570 (US), Gene 757 (UK and combined), Gene 698 (UK), and Gene 561 (US, and UK), reached statistical significance.

The most significant results were obtained for Gene 454, where 6 SNPs showed significant association with the phenotype at the allelic level

in the combined population and in one of the subpopulations. SNP H 1 showed highly significant results in the combined and US populations ($p=0.003$ for the allele test and $p=0.001$ for the genotype test in the combined population, 22% in control vs. 35% in cases, $p=0.0001$ in US for both tests, 20% in controls vs. 64% in cases). Two other SNPs had p -values <0.01 in the combined population: 1) SNP F -2 ($p=0.009$ for the allele test and $p=0.01$ for the genotype test in the combined population, 35% in controls vs. 23% in cases; $p=0.02$ in US, 37% in controls vs. 10 % in cases); and 2) SNP O 1 ($p=0.004$ for the allele test and $p=0.02$ for the genotype test in the combined population, 19% in controls vs. 9% in cases; $p=0.01$ and $p=0.04$ for the allele and genotype tests respectively in UK, 19% in controls vs. 8% in cases). Another SNP in exon O (O 6) had a p -value in the significant range ($p=0.02$ and $p<0.05$ for the allele and genotype tests respectively, in the combined population, 42% of controls vs. 31% of cases; $p=0.03$ for the allele test and $p=0.01$ for the genotype test in US, 39% of controls vs. 14% of cases; $p<0.05$ in UK for genotype test). In addition, two SNPs in high linkage disequilibrium with each other reached statistical significance in exon M: 1) M 1 ($p=0.01$ and $p=0.04$ for the allele and genotype tests, respectively, in the combined population, 42% in controls vs. 29% in cases; $p=0.003$ for the allele test and $p=0.02$ for the genotype test in US, 41% in controls vs. 6% in cases); and 2) M + 1 ($p=0.01$ for the allele test and $p=0.03$ for the genotype test in the combined population, 43% in controls vs. 31% in cases; $p=0.02$ and $p=0.01$ for the allele and genotype tests respectively, in US, 41% of controls vs. 14% of cases).

Gene 436 and Gene 214 both showed a single SNP that reached statistical significance in the combined population only. In Gene 436, which is adjacent to Gene 454, SNP K -2 was significant ($p=0.03$, 15% in controls vs. 7% of cases), while in Gene 214, SNP E +1 reached a similar level of significance ($p=0.03$, 28% in controls vs. 38% of cases).

For Gene 561, SNP Y +1 reached statistical significance in the UK population ($p=0.01$ for both the allele and genotype tests, 6% in controls vs.

no occurrence in cases) while SNP H 1 showed a significant genotype test in the US population ($p=0.04$).

A single SNP in Gene 698 showed a significant association with the total IgE subphenotype in the UK population ($p=0.01$ for the allele test and $p=0.02$ for the genotype test, 5% of controls vs. 13% of cases).

For Gene 757, SNP A +4 showed a significant genotype test in both the combined and the UK samples ($p<0.05$ combined, $p=0.03$ UK).

SNPs in two genes, Gene 515 and Gene 570, had significant genotype p-values in the US population alone (515 A 1, $p=0.02$; 515 A 2, $p=0.02$; 515 A 4; $p=0.03$; 570 F 1, $p=0.04$).

d. Specific IgE: The analyses were performed using asthmatic children with elevated specific IgE levels for at least one allergen, as described in the Linkage Analysis section (Example 3). First, sibling pairs were identified where both sibs were affected and satisfied this new criteria. Of these pairs, one sib was included in the case/control analyses if they showed evidence of linkage at the gene of interest. This phenotype was more restrictive than the Asthma yes/no criteria; hence the number of cases included in the analyses was reduced by approximately 38%.

The significance levels (p-values) for allelic association of the typed SNPs to the specific IgE phenotype are plotted in Figure 17 (combined population) and Figure 18 (US and UK populations, separately). Frequencies and p-values for SNPs associated with the specific IgE phenotype are shown in Tables 15A, 15B, and 15C for the combined population and for the UK and US populations, separately.

TABLE 15A: ASSOCIATION ANALYSIS OF SPECIFIC IgE PHENOTYPE COMBINED US/UK POPULATIONS

Combined US and UK						
GENE EXON	FREQUENCIES				ALLELE	GENOTYPE
	CNTL	N	CASE	N	P-VALUE	P-VALUE
454 B 1	7.1%	183	4.4%	57	0.3858	0.3685
454 E -1	25.8%	213	27.5%	69	0.7385	0.8563
454 E 1	0.8%	179	2.2%	69	0.3551	0.3524
454 E 2	49.1%	217	59.2%	71	0.0422	0.0191

454 F -2	34.6%	211	24.3%	70	0.0279	0.0078
454 G -1	8.4%	215	8.7%	69	0.8621	0.3087
454 H 1	22.1%	204	37.5%	68	0.0006	0.0003
454 H 2	2.0%	198	3.6%	70	0.3392	0.3340
454 K 1	1.9%	215	3.6%	70	0.3240	0.3186
454 L -1	6.7%	217	7.4%	68	0.8457	0.9252
454 M 1	42.3%	208	29.5%	61	0.0115	0.0316
454 M 2	6.8%	212	8.0%	69	0.7032	0.8568
454 M +1	43.2%	212	30.7%	70	0.0097	0.0245
454 O 1	19.1%	215	11.7%	64	0.0625	0.1300
454 O 3	17.6%	216	19.6%	69	0.6122	0.3714
454 O 5	3.0%	215	1.5%	65	0.5384	0.5325
454 O 6	42.4%	198	31.3%	67	0.0248	0.0468
436 A 1	1.2%	203	1.5%	65	0.6786	0.6956
436 C -1	15.3%	216	15.0%	70	1.0000	0.8439
436 D 1	5.0%	212	2.9%	70	0.3531	0.5945
436 E 1	46.3%	214	40.6%	69	0.2791	0.1703
436 G 1	10.4%	212	11.9%	67	0.6320	0.6418
436 K -2	14.9%	204	9.6%	68	0.1478	0.2662
436 K +1	4.7%	200	2.9%	69	0.4675	0.7077
436 L -1	4.4%	217	4.9%	71	0.8164	0.8123
436 L 1	1.9%	214	1.4%	70	1.0000	1.0000
515 A 1	43.1%	211	39.1%	64	0.4746	0.4804
515 A 2	37.2%	211	35.2%	64	0.7536	0.7931
515 A 3	7.4%	217	7.1%	70	1.0000	1.0000
515 A 4	43.5%	208	40.8%	65	0.6126	0.3468
515 A 5	4.1%	207	1.5%	66	0.2720	0.1767
515 A 7	2.4%	213	0.8%	67	0.4735	0.4692
570 C 2	9.5%	215	10.4%	53	0.8545	0.8669
570 C 4	9.5%	217	9.6%	52	1.0000	1.0000
570 F 1	47.9%	215	48.1%	52	1.0000	0.7607
757 A 2	18.1%	210	26.7%	58	0.0486	0.1177
757 A 4	1.6%	218	5.1%	59	0.0381	0.0362
757 A +4	39.4%	216	39.3%	61	1.0000	0.2242
698 E 1	6.9%	217	10.7%	61	0.1808	0.2696
698 I +1	32.5%	209	35.6%	59	0.5802	0.7116
561 P 1	34.0%	209	37.3%	59	0.5127	0.1175
561 J 1	14.9%	208	12.3%	61	0.5571	0.7970
561 H 1	9.0%	212	13.6%	59	0.1635	0.1228
561 E 1	0.0%	178	1.0%	50	0.2193	0.2193
561 C 1	0.2%	217	0.8%	60	0.3866	0.3869
561 B +1	13.4%	212	15.7%	51	0.5276	0.6102
561 B 1	48.3%	210	43.1%	51	0.3773	0.6088
561 Y +1	4.7%	212	1.7%	59	0.1892	0.1797
561 X -3	31.3%	214	31.1%	61	1.0000	0.8639
581 F +2	24.3%	216	21.2%	66	0.4850	0.7321
722 C 1	33.0%	209	30.3%	66	0.5949	0.5842
722 F +1	1.4%	217	1.5%	66	1.0000	1.0000
702 A -1	7.0%	215	5.4%	65	0.6871	0.8700

702 C 1	49.3%	213	42.4%	66	0.1948	0.2350
702 D 1	16.1%	217	13.6%	66	0.5835	0.7346
702 F 1	3.7%	217	6.8%	66	0.1457	0.1282
702 I 1	18.6%	204	18.9%	61	1.0000	0.9620
702 I 3	0.7%	217	0.8%	66	1.0000	1.0000
214 B 1	17.8%	214	21.4%	70	0.3816	0.1069
214 E -1	48.8%	202	51.5%	65	0.6147	0.4246
214 E +1	28.3%	217	36.4%	70	0.0732	0.1699

**TABLE 15B: ASSOCIATION ANALYSIS OF SPECIFIC IgE PHENOTYPE
UK POPULATION**

UK population		FREQUENCIES				ALLELE	GENOTYPE
GENE	EXON	CNTL	N	CASE	N	P-VALUE	P-VALUE
454	B 1	7.3%	109	4.9%	41	0.6057	0.5919
454	E -1	25.2%	137	29.8%	52	0.3638	0.5956
454	E 1	1.0%	104	2.9%	51	0.3357	0.3319
454	E 2	51.4%	140	59.4%	53	0.1712	0.0812
454	F -2	33.2%	137	27.4%	53	0.3250	0.1607
454	G -1	8.3%	138	9.6%	52	0.6859	0.4084
454	H 1	23.0%	135	32.0%	50	0.0817	0.0497
454	H 2	2.3%	131	2.9%	52	0.7176	0.7151
454	K 1	2.5%	138	3.9%	52	0.5014	0.4963
454	L -1	5.7%	140	6.9%	51	0.6346	0.6851
454	M 1	42.9%	133	34.8%	46	0.1789	0.2977
454	M 2	5.9%	136	7.8%	51	0.4836	0.6185
454	M +1	44.1%	136	35.6%	52	0.1606	0.1308
454	O 1	19.2%	138	12.0%	46	0.1520	0.3074
454	O 3	16.9%	139	19.6%	51	0.5458	0.5276
454	O 5	2.5%	139	2.1%	48	1.0000	1.0000
454	O 6	44.4%	124	36.0%	50	0.1866	0.0843
436	A 1	1.8%	135	2.1%	48	1.0000	0.7112
436	C -1	13.7%	139	16.3%	52	0.5150	0.7671
436	D 1	3.7%	136	3.9%	52	1.0000	1.0000
436	E 1	45.3%	137	43.1%	51	0.7279	0.1848
436	G 1	9.8%	138	12.0%	50	0.5669	0.7893
436	K -2	13.0%	131	9.8%	51	0.4765	0.8412
436	K +1	2.8%	123	3.9%	51	0.7370	0.7329
436	L -1	4.6%	140	4.7%	53	1.0000	1.0000
436	L 1	1.5%	137	1.0%	52	1.0000	1.0000
515	A 1	45.9%	135	39.4%	47	0.2805	0.4975
515	A 2	39.6%	135	35.1%	47	0.4624	0.5032
515	A 3	7.5%	140	9.6%	52	0.5288	0.5106
515	A 4	45.6%	136	41.2%	51	0.4841	0.7514
515	A 5	3.4%	133	2.0%	50	0.7340	0.7298
515	A 7	1.8%	138	1.0%	50	1.0000	1.0000
570	C 2	8.7%	138	11.9%	42	0.3960	0.4214
570	C 4	8.6%	140	11.0%	41	0.5152	0.5345

570 F 1	49.3%	138	46.4%	42	0.7088	0.8441
757 A 2	17.2%	137	26.1%	44	0.0869	0.0746
757 A 4	1.8%	140	5.6%	45	0.0678	0.0650
757 A +4	40.3%	139	40.4%	47	1.0000	0.1374
698 E 1	5.4%	140	12.8%	47	0.0217	0.0283
698 I +1	38.4%	133	36.7%	45	0.8028	0.9428
561 P 1	33.0%	135	38.9%	45	0.3087	0.2299
561 J 1	13.3%	132	10.6%	47	0.5897	0.5133
561 H 1	8.6%	139	9.6%	47	0.8341	0.8259
561 E 1	0.0%	110	0.0%	39	1.0000	1.0000
561 C 1	0.4%	140	0.0%	46	1.0000	1.0000
561 B +1	15.0%	137	16.2%	37	0.8554	0.9302
561 B 1	52.2%	135	43.2%	37	0.1905	0.3461
561 Y +1	5.5%	136	0.0%	46	0.0152	0.0133
561 X -3	33.0%	138	31.9%	47	0.8992	0.6051
581 F +2	24.6%	140	22.1%	52	0.6870	0.9085
722 C 1	35.0%	133	29.8%	52	0.3918	0.5778
722 F +1	1.8%	139	1.0%	52	1.0000	1.0000
702 A -1	7.2%	138	5.9%	51	0.8196	0.8126
702 C 1	47.8%	136	42.3%	52	0.3570	0.6439
702 D 1	17.9%	140	14.4%	52	0.5402	0.6574
702 F 1	2.9%	140	6.7%	52	0.1328	0.0644
702 I 1	18.3%	131	17.0%	47	0.8760	1.0000
702 I 3	1.1%	140	1.0%	52	1.0000	1.0000
214 B 1	19.2%	138	22.1%	52	0.5656	0.1256
214 E -1	47.9%	140	55.3%	47	0.2340	0.2996
214 E +1	30.7%	140	40.4%	52	0.0880	0.1988

**TABLE 15C: ASSOCIATION ANALYSIS OF SPECIFIC IgE PHENOTYPE
US POPULATION**

US population						
GENE EXON	FREQUENCIES				ALLELE	GENOTYPE
	CNTL	N	CASE	N	P-VALUE	P-VALUE
454 B 1	6.8%	74	3.1%	16	0.6918	0.6812
454 E -1	27.0%	76	20.6%	17	0.5205	0.6956
454 E 1	0.7%	75	0.0%	18	1.0000	1.0000
454 E 2	44.8%	77	58.3%	18	0.1940	0.3576
454 F -2	37.2%	74	14.7%	17	0.0144	0.0720
454 G -1	8.4%	77	5.9%	17	1.0000	1.0000
454 H 1	20.3%	69	52.8%	18	0.0002	0.0006
454 H 2	1.5%	67	5.6%	18	0.1974	0.1956
454 K 1	0.7%	77	2.8%	18	0.3439	0.3447
454 L -1	8.4%	77	8.8%	17	1.0000	0.7920
454 M 1	41.3%	75	13.3%	15	0.0034	0.0214
454 M 2	8.6%	76	8.3%	18	1.0000	0.8025
454 M +1	41.4%	76	16.7%	18	0.0066	0.0220
454 O 1	18.8%	77	11.1%	18	0.3355	0.7150
454 O 3	18.8%	77	19.4%	18	1.0000	0.7780

454 O 5	4.0%	76	0.0%	17	0.5942	0.5880
454 O 6	39.2%	74	17.6%	17	0.0177	0.0437
436 A 1	0.0%	68	0.0%	17	1.0000	1.0000
436 C -1	18.2%	77	11.1%	18	0.4578	0.8790
436 D 1	7.2%	76	0.0%	18	0.1275	0.3513
436 E 1	48.0%	77	33.3%	18	0.1373	0.2901
436 G 1	11.5%	74	11.8%	17	1.0000	0.8232
436 K -2	18.5%	73	8.8%	17	0.2097	0.7103
436 K +1	7.8%	77	0.0%	18	0.1272	0.3516
436 L -1	3.9%	77	5.6%	18	0.6478	0.6439
436 L 1	2.6%	77	2.8%	18	1.0000	1.0000
515 A 1	38.2%	76	38.2%	17	1.0000	0.0236
515 A 2	32.9%	76	35.3%	17	0.8414	0.0275
515 A 3	7.1%	77	0.0%	18	0.1286	0.1169
515 A 4	39.6%	72	39.3%	14	1.0000	0.0307
515 A 5	5.4%	74	0.0%	16	0.3538	0.3418
515 A 7	3.3%	75	0.0%	17	0.5860	0.5798
570 C 2	11.0%	77	4.5%	11	0.7043	0.7580
570 C 4	11.0%	77	4.5%	11	0.7043	1.0000
570 F 1	45.5%	77	55.0%	10	0.4796	0.1204
757 A 2	19.9%	73	28.6%	14	0.3178	0.1027
757 A 4	1.3%	78	3.6%	14	0.3924	0.3942
757 A +4	37.7%	77	35.7%	14	1.0000	1.0000
698 E 1	9.7%	77	3.6%	14	0.4729	0.7729
698 I +1	22.4%	76	32.1%	14	0.3337	0.2961
561 P 1	35.8%	74	32.1%	14	0.8304	0.2910
561 J 1	17.8%	76	17.9%	14	1.0000	0.8292
561 H 1	9.6%	73	29.2%	12	0.0142	0.0197
561 E 1	0.0%	68	4.5%	11	0.1392	0.1392
561 C 1	0.0%	77	3.6%	14	0.1538	0.1538
561 B +1	10.7%	75	14.3%	14	0.5260	0.5082
561 B 1	41.3%	75	42.9%	14	1.0000	1.0000
561 Y +1	3.3%	76	7.7%	13	0.2714	0.2702
561 X -3	28.3%	76	28.6%	14	1.0000	1.0000
581 F +2	23.7%	76	17.9%	14	0.6274	0.3029
722 C 1	29.6%	76	32.1%	14	0.8239	0.7629
722 F +1	0.6%	78	3.6%	14	0.2819	0.2826
702 A -1	6.5%	77	3.6%	14	1.0000	1.0000
702 C 1	52.0%	77	42.5%	14	0.4162	0.0825
702 D 1	13.0%	77	10.7%	14	1.0000	1.0000
702 F 1	5.2%	77	7.1%	14	0.6533	0.6486
702 I 1	19.2%	73	25.0%	14	0.4519	0.6731
702 I 3	0.0%	77	0.0%	14	1.0000	1.0000
214 B 1	15.1%	76	19.4%	18	0.6124	0.5757
214 E -1	50.8%	62	41.7%	18	0.3509	0.4623
214 E +1	24.0%	77	25.0%	18	1.0000	1.0000

For the specific IgE subphenotype, SNPs in Gene 454 and Gene 757 showed a significant association in the combined population when comparing the allele frequencies in the case and control groups. When analyzing the populations separately, SNPs in Gene 561 showed a significant association in both the US and UK populations. In addition, five SNPs in Gene 454 showed association with the subphenotype in the US population. Gene 698 contained a SNP reaching statistical significance in the UK population only. Additional significant results were identified when comparing the genotype frequencies in the case and control groups. SNPs in Gene 515, Gene 561, and Gene 454 reached statistical significance in the US population. SNPs in Gene 454, Gene 561, and Gene 698 were significant in the UK and in the combined population. In addition, a SNP in gene 757 was significant at the 0.05 level in the combined population.

The most significant results were found in Gene 454, where 6 SNPs yield significant association with the subphenotype in the combined population. SNP H 1 showed highly significant results in the combined and US populations ($p=0.0006$ and 0.0003 for the allele and genotype tests respectively in the combined population, 22% in control vs. 38% in cases; $p=0.0002$ for the allele test and $p=0.0006$ for the genotype test in the US population, 20% in controls vs. 53% in cases; genotypic test $p<0.05$ in the UK population). Two SNPs in exon M gave significant results: 1) M 1 ($p=0.01$ and $p=0.03$ for the allele and genotype tests in the combined population, 42% in controls vs. 30% in cases; $p=0.003$ for the allele and $p=0.02$ for the genotype test in US, 41% in controls vs. 13% in cases); and 2) M + 1 ($p=0.01$ and $p=0.02$ for the allele and genotype tests respectively, in the combined population, 43% in controls vs. 31% in cases; $p=0.007$ for the allele and $p=0.02$ for the genotype test in US, 41% of controls vs. 17% of cases). Three other SNPs had p -values <0.05 in the combined population: 1) SNP E 2 ($p=0.04$ and $p=0.02$ for the allele and genotype tests respectively, in the combined population, 49% in controls vs. 59% in cases); 2) SNP F -2 ($p=0.03$ and $p=0.008$ for the allele and genotype tests

respectively, in the combined population, 35% in controls vs. 24% in cases; $p=0.01$ for the allele test in US, 37% in controls vs. 15 % in cases); and 3) SNP O 6 ($p=0.02$ and $p<0.05$ for the allele and genotype tests respectively, in the combined population, 42% in controls vs. 31% in cases; $p=0.02$ for the allele test and $p=0.04$ for the genotype test in US, 39% in controls vs. 18% in cases).

For Gene 561, SNP Y +1 reached statistical significance in the UK population ($p=0.02$ and $p=0.01$ for the allele and genotype tests respectively, 6% in controls vs. no occurrence in cases) while SNP H 1 had a significant p-value in the US population ($p=0.01$ and $p=0.02$ for the allele and genotype tests respectively, 10% in controls vs. 29% in cases).

A single SNP in Gene 698 showed a significant association with the specific IgE subphenotype in the UK population ($p=0.02$ and $p=0.03$ for the allele and genotype tests respectively, 5% of controls vs. 13% of cases).

For Gene 757, SNPs A 2 and A 4 showed a significant association with the subphenotype in the combined population (A 2 $p<0.05$, 18% in controls vs. 27% in cases; A 4 $p=0.04$ for both the allele and genotype tests, 2% in controls vs. 5% in cases).

Additionally, three SNPs in Gene 515 had significant genotype p-values in the US population alone (A 1, $p=0.02$; A 2, $p=0.03$; A 4; $p=0.03$).

In summary, evidence obtained from association studies implicated several genes in the 12q23-ter region as being involved in respiratory diseases. This was supported by analysis of the asthma (yes/no) phenotype, BHR phenotype, total IgE phenotype, and specific IgE phenotype in asthmatic individuals. Thus, chromosome 12q23-ter encompassed genes involved in asthma and related diseases thereof.

EXAMPLE 12: HAPLOTYPE ANALYSES

In addition to the analysis of individual SNPs, haplotype frequencies between the case and control groups were also compared. The haplotypes were constructed using a maximum likelihood approach. Since existing software for predicting haplotypes was unable to utilize individuals with

missing data, a program was developed to analyze all individuals. This provided more accurate haplotype frequency estimates. Haplotype analysis based on multiple SNPs in a gene was expected to provide increased evidence for an association between a given phenotype and that gene if all
 5 haplotyped SNPs were involved in the manifestation of the phenotype. In other words, allelic variation involving the haplotyped SNPs was expected to be associated with different risks of or susceptibilities to the phenotype.

The estimated frequency of each haplotype was compared between cases and controls by a permutation test. An overall comparison of the
 10 distribution of all haplotypes between the two groups was also performed. For each gene with two SNPs or more, all 2-at-a-time haplotypes were constructed, and their frequencies were compared between the case and control groups. P-values for the overall comparisons were plotted against a coordinate system based on genomic sequence (average location of the two
 15 SNPs in the haplotype). This was used to visualize regions where haplotype association was present. A small p-value (or a large value of $-\log(p)$ as plotted in the figures described below) was indicative of an association between the haplotyped SNPs and the disease phenotype. The analysis was repeated for the US and UK population, separately, to adjust for the
 20 possibility of genetic heterogeneity.

1. Asthma phenotype: Figure 19 (combined population) and Figure 20 (US and UK populations separately) shows the results for the haplotype analysis (2-at-a-time) for all SNPs in Gene 214, Gene 436, Gene 454, Gene 515, Gene 561, Gene 570, Gene 698, Gene 702, Gene 722, and
 25 Gene 757.

The most significant associated haplotype was formed by SNPs E -1 and E +1 from Gene 214, which had a p-value of 0.00001 in the combined population ($p=0.00002$ in UK, non-significant in US). This SNP combination was much more significant than the analysis of these SNPs alone
 30 (combined population $p=0.04$ for E +1 and $p = 0.93$ for E -1). Eighteen SNP combinations had p-values < 0.01 in gene 454 in the combined population,

with the most significant haplotype consisting of SNP E 2 and F -2. This haplotype had a p-value of 0.001 in the combined population ($p=0.008$ in the US, $p<0.05$ in UK). Although this result was more significant than the analysis of these SNPs alone, the levels of significance found in the

5 haplotypes of Gene 454 were comparable to the significance obtained from the analysis of the SNPs alone (in the combined population: E 2 and M +1, $p=0.003$; G -1 and M +1, $p=0.004$; E -1 and E 2, $p=0.004$; E 2 and H 1, $p=0.004$; E 2 and O 6, $p=0.004$; E 2 and M 1, $p=0.004$; H 1 and O 3, $p=0.005$; E 1 and E 2, $p=0.005$; B 1 and H 1, $p=0.006$; E 1 and H 1, $p=0.006$;

10 $p=0.006$; E 1 and M +1, $p=0.007$; E 1 and F -2, $p=0.007$; B 1 and E 2, $p=0.007$; G -1 and H 1, $p=0.008$; H 1 and O 1, $p=0.008$; F -2 and M 1, $p=0.009$; E 2 and G -1, $p=0.01$).

In Gene 561, a single haplotype (J 1 and H 1) reached statistical significance at the 0.01 level in the combined population ($p=0.008$), while all

15 seven haplotype combinations with SNP Y + 1 yield significant results at the 0.01 level in the UK population (P 1 and Y +1, $p=0.0006$; C 1 and Y +1, $p=0.0007$; E 1 and Y +1, $p=0.0008$; J 1 and Y +1, $p=0.001$; H 1 and Y +1, $p=0.001$; B +1 and Y +1, $p=0.002$; B 1 and Y +1, $p=0.002$; Y +1 and X -3, $p=0.004$). The SNP combination of H 1 and E 1 had a significant

20 association in the US population ($p=0.009$). In addition, in the combined population, the haplotypes formed by SNPs A2 and A +4 in gene 757 were more significantly associated with the disease ($p=0.004$) than any of these SNPs alone ($p=0.03$ for A 2, $p=0.60$ for A +4).

2. Bronchial Hyper-responsiveness: A similar test for association of 2-

25 SNP-at-a-time haplotypes with BHR ($PC_{20} \leq 16$ mg/ml) was performed. In Figures 21 and 22, the haplotype analysis (2-at-a-time) for all SNPs in Gene 214, Gene 436, Gene 454, Gene 515, Gene 561, Gene 570, Gene 698, Gene 702, Gene 722, and Gene 757 is shown for the combined population, and for the UK and the US populations, respectively.

30 The most significant associated haplotype was formed by SNPs E -1 and E +1 from Gene 214, which had a p-value of 0.00007 in the combined

population ($p=0.0002$ in UK, non-significant in US). Four SNP combinations had p -values <0.01 in Gene 454 in the combined population, (E -1 and E 2, $p=0.004$; E 1 and E 2, $p=0.004$; E 2 and G -1, $p=0.005$; E 2 and F -2, $p=0.008$), and one SNP combination in the UK (E 1 and E 2, $p=0.007$). In

5 Gene 561, four haplotypes reached statistical significance at the 0.01 level in the combined population (J 1 and H 1, $p=0.003$; E 1 and Y +1, $p=0.003$; J 1 and E 1, $p=0.006$; J 1 and Y +1, $p=0.009$), one in the UK population (J 1 and Y +1, $p=0.01$), and one in the US (H 1 and E 1, $p=0.002$). In addition, in the combined population, a haplotype formed by SNPs in Gene 757 (A2 and

10 A +4, $p=0.003$) was significant at the 0.01 level.

3. Total IgE: A similar test for association of 2-SNP-at-a-time haplotypes with elevated levels of total IgE was performed. In Figures 23 and 24, the haplotype analysis (2-at-a-time) for all SNPs in Gene 214, Gene 436, Gene 454, Gene 515, Gene 561, Gene 570, Gene 698, Gene 702, Gene 722, and

15 Gene 757 is shown for the combined and for the UK and the US populations, respectively.

The most significant associated haplotype was formed by SNPs E -1 and E +1 from Gene 214, with a p -value of 0.000003 in the combined population ($p=0.000005$ in UK, non-significant in US). Thirteen SNP

20 combinations had p -values <0.01 in gene 454 in the combined population (K 1 and O 1, $p=0.002$; H 1 and O 1, $p=0.002$; O 1 and O 3, $p=0.004$; E 1 and O 1, $p=0.005$; G -1 and H 1, $p=0.006$; H 1 and O 3, $p=0.007$; F -2 and M 1, $p=0.008$; H 2 and O 1, $p=0.008$; B 1 and O 1, $p=0.009$; M 1 and O 1, $p=0.009$; G -1 and M +1, $p=0.009$; E 2 and O 1, $p=0.009$; F -2 and H 1, $p=0.01$), one SNP combination in the UK (K 1 and O 1, $p=0.007$), and twenty-nine SNP combinations in the US (H 1 and K 1, $p=0.0001$; E 1 and H 1, $p=0.0002$; H 1 and H 2, $p=0.0002$; E 2 and H 1, $p=0.0003$; B 1 and H 1, $p=0.0003$; H 1 and M 1, $p=0.0003$; G -1 and H 1, $p=0.0004$; H 1 and O 5, $p=0.0004$; H 1 and O 3, $p=0.0004$; H 1 and O 6, $p=0.0005$; E -1 and H 1, $p=0.0006$; F -2 and H 1, $p=0.0006$; H 1 and M +1, $p=0.0006$; H 1 and O 1, $p=0.0007$; H 1 and L -1, $p=0.0008$; H 1 and M 2, $p=0.001$; M 1 and O 3,

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p=0.001; M 1 and O 5, p=0.002; H 2 and M 1, p=0.002; E 1 and M 1, p=0.002; K 1 and M 1, p=0.002; F -2 and M 1, p=0.004; L -1 and M 1, p=0.005; M 1 and M 2, p=0.005; B 1 and M 1, p=0.005; F -2 and G -1, p=0.005; M 1 and O 1, p=0.006; E 2 and M 1, p=0.006; M 1 and M +1, p=0.007). In Gene 561, three haplotypes reached statistical significance at the 0.01 level in the UK sample (E 1 and Y +1, p=0.008; C 1 and Y +1, p=0.008; H 1 and Y +1, p=0.009), and two reached statistical significance in the US sample (E 1 and C 1, p=0.004; E 1 and Y +1, p=0.004). In Gene 757, the haplotype formed with SNP A2 and SNP A +4 was significant at the 0.01 level in the combined population (p=0.002), and in the UK population (p=0.006).

4. Specific IgE: A similar test for association of 2-SNP-at-a-time haplotypes with elevated levels of specific IgE was performed. In Figures 25 and 26, the haplotype analysis (2-at-a-time) for all SNPs in Genes 214, 436, 454, 515, 561, 570, 698, 702, 722 and 757 is shown for the combined and for the UK and the US populations, respectively.

The most significant associated haplotype was formed by SNPs E -1 and E +1 from Gene 214, with a p-value of 0.000006 in the combined population (p=0.000003 in UK, non-significant in US). Sixteen SNP combinations had p-values <0.01 in gene 454 in the combined population (H 1 and O 3, p=0.0007; H 1 and K 1, p=0.002; G -1 and H 1, p=0.002; H 1 and O 1, p=0.003; E 2 and H 1, p=0.003; H 1 and H 2, p=0.003; E 1 and H 1, p=0.003; B 1 and H 1, p=0.003; H 1 and M 2, p=0.003; H 1 and O 5, p=0.004; H 1 and M +1, p=0.004; H 1 and L -1, p=0.004; F -2 and H 1, p=0.004; H 1 and M 1, p=0.005; E -1 and H 1, p=0.006; H 1 and O 6, p=0.007), and thirty-three SNP combinations in the US (H 1 and M 1, p=0.0005; E 1 and H 1, p=0.0006; H 1 and O 5, p=0.0007; H 1 and O 6, p=0.0008; H 1 and M +1, p=0.0009; H 1 and K 1, p=0.001; F -2 and H 1, p=0.001; K 1 and M 1, p=0.001; H 1 and H 2, p=0.001; M 1 and O 3, p=0.001; G -1 and H 1, p=0.001; H 1 and O 3, p=0.001; B 1 and H 1, p=0.001; H 1 and L -1, p=0.002; E -1 and H 1, p=0.002; E 2 and H 1,

p=0.002; H 1 and M 2, p=0.002; H 1 and O 1, p=0.002; M 1 and M +1, p=0.002; K 1 and M +1, p=0.003; M 1 and O 5, p=0.003; M +1 and O 5, p=0.004; E 2 and M 1, p=0.005; F -2 and K 1, p=0.005; E 1 and M 1, p=0.005; K 1 and O 6, p=0.006; M +1 and O 6, p=0.006; H 2 and M 1, p=0.007; F -2 and O 3, p=0.008; M +1 and O 3, p=0.008; E 2 and M +1, p=0.009; M 1 and O 1, p=0.009; O 5 and O 6, p=0.009). In Gene 561, two haplotypes reached statistical significance at the 0.01 level in the UK population (E 1 and Y +1, p=0.003; C 1 and Y +1, p=0.007), and two reached statistical significance in the US population (E 1 and C 1, p=0.007; H 1 and E 1, p=0.009). In Gene 757, the haplotype formed with SNP A2 and SNP A +4 was significant at the 0.01 level in the combined population (p=0.002) and in the UK sample (p=0.006).

In summary, haplotype analysis of the SNPs provided additional evidence demonstrating the presence of asthma susceptibility genes on chromosome 12. In some SNP combinations, the level of significance of the association was increased by an order of magnitude.

EXAMPLE 13: TRANSMISSION DISEQUILIBRIUM TEST (TDT)

A family based test of association, the transmission disequilibrium test (TDT), was conducted for Gene 454. By selecting a single affected offspring in each family, the TDT test performed a test of association (due to linkage disequilibrium) in the presence of linkage. The test determined whether a particular allele or genotype was preferentially transmitted to an affected individual over what would be expected by chance. Only heterozygote parents were considered informative for the TDT. In addition, to increase power, heterozygote parents transmitting a different allele to two affected offspring were ignored. Accordingly, the TDT would be based on the same families that contributed to the linkage signal. The significance levels were estimated by Markov Chain Monte Carlo simulation methods as implemented in TDTEX from the S.A.G.E. program (1997, Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve University,

Cleveland, OH). As only heterozygote parents contributed information to the TDT test, SNP haplotypes (all 2-at-a-time and all 3-at-a-time) were also constructed based on family data with the program GENEHUNTER (Kruglyak et al., 1996). This served to increase the informativeness of the single SNPs. These haplotypes were then used as "alleles" in future TDT analyses. In addition, p-values obtained from the TDT analyses were compared to the p-values obtained from the haplotyping in the case/control setting. To check for consistency, p-values, associated with testing frequencies in cases and controls, were examined when selecting the overtransmitted alleles or genotypes identified in the TDT test.

1. Asthma Phenotype: Three candidate SNPs for Gene 454 were typed in the extended population in order to investigate further the association seen in the case- control study. All three SNPs result in amino acid changes (E 2, histidine to tyrosine (C→T); H 1 and H 2, arginine to histidine (G→A)). Results are shown in Table 16. Column 1 lists the exon(s) containing the SNP(s) of interest. Column 2 lists the overtransmitted alleles or genotypes. Column 3 lists the TDT p-values. Columns 4, 5, and 6 list the p-values, the frequencies in the cases, and the frequencies in the controls of the overtransmitted alleles or genotypes, respectively.

Since the TDT was not influenced by admixture, it was performed using the combined US and UK populations. For SNPs E 2 and H 1, the genotype formed by the CA/CA haplotypes was significantly overtransmitted to the affected individuals ($p=0.04$). In addition, this genotype was found in only 2% of the controls while 12% of the cases harbor this genotype. This difference was highly significant ($p=0.0002$). For the SNP combination comprising H 1 and H 2, the AG/AG genotypes were overtransmitted to affected individuals. This result approached the statistical level of 0.05 ($p=0.06$). Moreover, this genotype was more frequent in the cases (14%) compared to the controls (2%), and this difference was highly significant ($p=0.00005$). The TDT results supported the association previously

observed in the case-control studies for Gene 454. The results also pointed to a recessive mechanism of transmission, as the genotype test showed the strongest evidence of association.

TABLE 16: TDT ANALYSIS OF ASTHMA PHENOTYPE

Asthma Yes/No					
Combined US and UK					
Exon	Over-Transmitted Allele	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 E 2	T	1.0000	0.0058	50.9%	39.7%
454 H 1	A	0.3484	0.0032	22.1%	33.0%
454 H 2	G	0.1094	0.7801	98.0%	97.4%
454 E 2 H 1	CA	0.3874	0.0008	15.4%	27.6%
454 E 2 H 2	CG	0.4612	0.0097	48.4%	59.4%
454 H 1 H 2	AG	0.0900	0.0036	20.0%	30.4%
454 E 2 H 1 H 2	CAG	0.2167	0.0015	15.3%	26.8%
Exon	Over-Transmitted Genotype	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 E 2	TT	0.8375	0.0070	28.6%	13.7%
454 H 1	AA	0.1057	0.0022	4.9%	17.0%
454 H 2	GG	0.1107	0.7776	96.0%	94.8%
454 E 2 H 1	CA/CA	0.0359	0.0002	1.5%	11.6%
454 E 2 H 2	CG/CG	0.2829	0.2477	26.8%	33.0%
454 E 2 H 2	TG/TG	0.2829	0.0038	26.3%	12.2%
454 H 1 H 2	AG/AG	0.0637	0.00005	2.0%	14.3%
454 E 2 H 1 H 2	CAG/CAG	0.0877	0.0001	1.0%	10.7%

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2. Bronchial Hyper-responsiveness: The TDT analyses were repeated using only the asthmatic pairs that satisfied the additional criteria of having a $PC_{20} \leq 16$ mg/ml (Table 17). As for the case of the asthma yes/no phenotype, significance was reached with the genotypic TDT test.
- 10 For this subphenotype, genotype AA of SNP H 1 was overtransmitted to affected individuals ($p=0.04$). This genotype was also present more often in the cases than in the controls (17% cases, 5% controls, $p=0.02$). Two haplotype combinations had overtransmitted genotypes that approached statistically significant levels: genotype CA/CA for SNPs E 2 and H 1
- 15 ($p=0.06$) and genotype CAG/CAG for SNPs E2, H1 and H2 ($p=0.06$). Both

of these genotypes were found more often in the cases (CA/CA 13%, CAG/CAG 11%) than in the controls (CA/CA 2%, CAG/CAG 1%), and these differences were highly significant ($p=0.0008$ for CA/CA, $p=0.0014$ for CAG/CAG).

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TABLE 17: TDT ANALYSIS OF BHR PHENOTYPE

Combined US and UK					
Exon	Over-Transmitted Allele	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 E 2	C	1.0000	0.0074	49.1%	63.6%
454 H 1	A	0.7974	0.0223	22.1%	33.0%
454 H 2	G	0.6252	0.2962	98.0%	96.3%
454 E 2 H 1	CA	0.7986	0.0037	15.4%	28.8%
454 E 2 H 2	CG	0.7156	0.0274	48.4%	61.4%
454 H 1 H 2	AG	0.5338	0.0442	20.0%	29.3%
454 E 2 H 1 H 2	CAG	0.9090	0.0192	15.3%	26.4%
Exon	Over-Transmitted Genotype	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 E 2	TT	0.7917	0.0140	28.6%	10.9%
454 H 1	AA	0.0429	0.0181	4.9%	17.0%
454 H 2	GG	0.6235	0.2923	96.0%	92.6%
454 E 2 H 1	CA/CA	0.0601	0.0008	1.5%	13.2%
454 E 2 H 2	CG/CG	0.7211	0.2373	26.8%	35.2%
454 H 1 H 2	AG/AG	0.1319	0.0022	2.0%	13.2%
454 E 2 H 1 H 2	CAG/CAG	0.0632	0.0014	1.0%	11.3%

3. Total IgE The TDT analyses were also performed using the phenotype previous described for total IgE (Table 18). Again, significance was reached with the genotypic TDT test. For this subphenotype, genotype AA of SNP H 1 was overtransmitted to affected individuals ($p=0.03$). This genotype was also present more often in the cases than in the controls (21% cases, 5% controls, $p=0.0001$). Two genotypes for the SNP combination formed by E2 and H1 had statistically significant overtransmission: genotype CA/CA and genotype CA/TA ($p<0.05$). Both genotypes were found more often in the cases (CA/CA 12%, CA/TA 9%)

than in the controls (CA/CA 2%, CA/TA 3%), and these differences were significant ($p=0.0009$ for CA/CA, $p=0.03$ for CA/CT).

TABLE 18: TDT ANALYSIS OF TOTAL IgE PHENOTYPE

Combined US and UK					
Exon	Over-Transmitted Allele	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 E 2	C	1.0000	0.0552	49.1%	58.3%
454 H 1	A	0.0821	0.0030	22.1%	35.3%
454 H 2	G	0.2896	0.2146	98.0%	95.8%
454 E 2 H 1	CA	0.5439	0.0040	15.4%	27.3%
454 E 2 H 2	CG	0.6807	0.1055	48.4%	56.8%
454 H 1 H 2	AG	0.3460	0.0116	20.0%	30.9%
454 E 2 H 1 H 2	CAG	0.3447	0.0101	15.3%	25.8%
Exon	Over-Transmitted Genotype	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 H 1	AA	0.0349	0.0001	4.9%	20.6%
454 H 2	GG	0.2888	0.2088	96.0%	91.6%
454 E 2 H 1	CA,CA	0.0477	0.0009	1.5%	11.8%
454 E 2 H 1	CA,TA	0.0477	0.0314	2.5%	8.8%
454 E 2 H 2	CG,CG	0.7049	0.8766	26.8%	28.2%
454 H 1 H 2	AG,AG	0.1707	0.00009	2.0%	16.2%
454 E 2 H 1 H 2	CAG,CAG	0.1457	0.0013	1.0%	10.3%

- 5 4. Specific IgE: The TDT analyses were performed using the phenotype previous described for specific IgE (Table 19). There were no alleles or genotypes that were significantly overtransmitted at the 0.05 level. However, the test for the overtransmission of genotype AA SNP H 1 had a p -value <0.1 . This genotype was present more often in the cases than in
- 10 the controls (22% cases, 5% controls, $p=0.0003$).

TABLE 19: TDT ANALYSIS OF SPECIFIC IgE PHENOTYPE

Combined US and UK					
Exon	Over-Transmitted Allele	TDT p-value	Case/Control p-value	Control Freq	Case Freq
454 H 1	A	0.1555	0.00006	22.1%	37.5%
454 H 2	G	0.3757	0.3392	98.0%	96.4%
454 E 2 H 1	CA	0.7101	0.0006	15.4%	30.2%
454 E 2 H 2	TG	0.8317	0.0332	49.5%	38.6%

454 H 1 H 2	AG	0.1369	0.0012	20.0%	33.9%
454 E 2 H 1 H 2	CAG	0.6602	0.0012	15.3%	29.3%
	Over-Transmitted	TDT	Case/Control	Control	Case
Exon	Genotype	p-value	p-value	Freq	Freq
454 H 1	AA	0.0910	0.00003	4.9%	22.1%
454 H 2	GG	0.3740	0.3340	96.0%	92.9%
454 E 2 H 1	CA/TA	0.2586	0.0314	2.5%	8.8%
454 E 2 H 2	TG/TG	0.7369	0.0118	26.3%	11.4%
454 H 1 H 2	AG/AG	0.1104	0.00003	2.0%	17.7%
454 E 2 H 1 H 2	CAG/CAG	0.3841	0.0004	1.0%	11.8%
454 E 2 H 1 H 2	CGA/CGA	0.3841	1.0000	0.0%	0.0%

EXAMPLE 14: GENE ANALYSIS AND POTENTIAL FUNCTION

1. Functional Role of Gene 454 in Asthma and Related Diseases

Extracellular ATP triggers a variety of responses in several cell types, including contraction of smooth muscles, regulation of nitric oxide production from endothelium, stimulation of cytokine release from immune cells, and modulation of several other metabolic pathways. The receptors that mediate these diverse effects are the P2 purinoreceptors, which are divided into two subgroups: P2Y and P2X receptors. The P2X receptors are a family of multimeric ligand-gated ion channels activated solely by extracellular ATP and structurally distinct from other ligand-gated channels.

Gene 454 represents the seventh member of the P2X receptor family, P2X7. The nucleic acid sequence of Gene 454 corresponds to SEQ ID NO:19, and the encoded amino acid sequence corresponds to SEQ ID NO:111, as disclosed herein (see Figures 7A-7H). The Gene 454 transcript is 5.087 Kb, the gene is ~55 Kb in size, and includes 13 exons. The Gene 454 ORF is 1788 bp long and encodes a 596 amino acid protein. The 5' and 3' untranslated regions are 69 bp and 3230 bp in length, respectively. As determined by the experiments described herein, Gene 454 is expressed in brain, heart, skeletal muscle, spleen, kidney, liver, placenta, lung, leukocytes, lymph and fetal liver tissues (Figure 6).

Data have indicated that the P2X7 receptor is involved in cell death, cytokine release, and the shedding of surface antigens. The P2X7 receptor

also mediates activation of the transcription factors NF-K-beta and NFAT. The P2X7 receptor displays unique permeability properties. At low ATP concentrations P2X7 forms small ATP-gated cation channels, allowing the influx of small cations, including Ca^{2+} , into the intracellular environment.

- 5 Notably, in rat peritoneal mast cells, there is a direct correlation between the influx of Ca^{2+} and the release of histamine as a consequence of ATP levels (Schulman et al., 1999, *Am. J. Respir. Cell Mol. Biol.* 20:530-537). In addition, at these levels of ATP, various proteases are activated including membrane metalloproteases and intracellular caspases (Gu et al., 1998, 10 *Blood* 92:946-951).

- At high ATP concentrations, the P2X7 receptor pore size increases allowing the passage of anions as well as cations up to 900 daltons in size (Nihei et al., 2000, *Mem. Inst. Oswaldo Cruz* 95:415-428). Interestingly, inhalation of aerosolized ATP has been shown to trigger bronchoconstriction 15 in healthy and asthmatic individuals. In asthmatics, ATP was 50 times more potent than methacholine, and 87-fold more potent than histamine, in producing a 15% decrease in FEV_1 . (Schulman et al., 1999, *Am. J. Respir. Cell Mol. Biol.* 20:530-537). This suggests that extracellular ATP acts as an important modulator of pro-inflammatory regulation via the P2X7 receptor.

- 20 The P2X7 protein contains two transmembrane domains connected by a large extracellular loop, and intracellular N-terminal and C-terminal domains (Figure 10). P2X7 shares significant amino acid identity with the other members of the P2X receptor family (30-40%), except in the C-terminus domain, which is 240 amino acids long. P2X7 contains a long 25 unique carboxyl terminus, which appears to be involved in the permeability properties of the P2X7 receptor. Truncation of the cytoplasmic tail abolishes ATP-induced uptake of the fluorescent dye YoPro-1 and ethidium bromide (Gu et al., 2001, *JBC* 276:11135-11142). Further, a SNP (A→C) in the cytoplasmic tail was identified in the Caucasian population. The SNP results 30 in a glutamic acid to alanine change at amino acid 496. This amino acid substitution results in a loss of functional P2X7 in homozygotes, and results

a 50% loss of function in heterozygotes (Gu et al., 2001, *JBC* 276:11135-11142). The expression of P2X7 has been observed mainly in cells of the immune and hematopoietic system, and P2X7 has been shown to mediate the ATP-induced apoptotic death in monocytes, macrophages, and lymphocytes. However, P2X7 expression has been observed in other cell-types at lower levels. In particular, fibroblasts express P2X7, and are responsive to ATP.

Fibroblasts are non-excitabile cells that play a role in the modulation of a variety of microenvironmental situations to which these cells are exposed. In the lung, fibroblasts lie in the lamina propria under the basement membrane. The bronchial epithelium lies above the basement membrane, and is attached thereto. In accordance with one model of respiratory diseases, allergens cause the cells of the bronchial epithelium to release their cytoplasmic contents. The cellular ATP concentration of each cell is estimated to be 5-10 mM. The released ATP immediately passes through the basement membrane by passive diffusion. This triggers the P2X7 receptors on the surface of the fibroblast cells to dilate, forming an open channel. The P2X7 receptors allow the influx of cations and anions up to 900 daltons. One of these ions triggers a signal transduction cascade that induces the final step in the post-translational processing of pro-IL-1 β , a multipotential inflammatory mediator (Solle et al., *JBC* 276:125-132). The mature IL-1 β binds to receptors on target cells that elicit signaling cascades. This leads to the up-regulation of gene products such as matrix metalloproteases, cyclooxygenase-2, IL-6 and cellular adhesion molecules, which contribute to inflammation.

IL-6 is an important pro-inflammatory cytokine that is secreted by mononuclear phagocytes, antigen-presenting cells, and fibroblasts. In accordance with the current knowledge in the art, secretion of IL-6 creates a pro-inflammatory microenvironment that induces the release of other factors such as growth factors, cytokines, and prostaglandins. This, in turn, enhances the stimulation and propagation of fibroblasts, and leads to an

increase in the release of pro-inflammatory molecules. Fibroblasts also play a role in exuding extracellular matrix. Notably, in asthmatics, the basement membrane is thicker than in normal individuals due to the abnormal repair of the bronchial epithelium by fibroblasts. Further, myofibroblasts are also in abundance in asthmatic individuals, due in part to pro-inflammatory stimulation.

The Gene 454 SNPs (Table 10; Figures 7A-7H, and 10) identified by the experiments described herein result in nucleotide changes that may disrupt the intracellular function, stability, splicing, or expression of the encoded protein. It is possible that the nucleotide changes cause an increase or decrease in the normal activities or levels the P2X7 receptor, thereby affecting the pro-inflammatory response triggered by ATP, and resulting in asthmatic symptoms. The sum of these data indicates that Gene 454 (P2X7) is involved in the pathophysiology of respiratory disorders, including asthma.

2. Functional Role of Gene 561 in Asthma and Related Diseases

Gene 561 is the human ortholog of rat RIMBP2, a scaffold protein. RIMBP2 protein binds to RIM, a putative effector of Rab3, and appears to recruit synaptic vesicles by a tethering reaction. RIMBP2 is an intracellular protein that contains an SH3 domain, which is thought to be involved in binding to RIM. RIMBP2 also contains fibronectin type III repeats, which are rarely observed in intracellular proteins (Wang et al., 2000, *JBC* 275:20033-2044).

The nucleotide sequence of Gene 561.1 alternate splice variant (also referred to as 561.nt1), corresponds to SEQ ID NO:31 (Figures 27A-27K), and the encoded amino acid sequence (also referred to as Gene 561.aa1) corresponds to SEQ ID NO:120. The nucleotide sequence of Gene 561.2 alternate splice variant (also referred to as 561.nt2) corresponds to SEQ ID NO:32 (Figures 28A-28C), and the encoded amino acid sequence (also referred to as Gene 561.aa2) corresponds to SEQ ID NO:121. As determined by the experiments described herein, the transcript size of

Gene561.nt1 and Gene561.nt2 is 7.9 and 6.1 Kb, respectively (Figure 8). An alternative splice site has been identified at a position in the 3'UTR, between exons J and I (Figure 8). RT-PCR data indicate that Gene 561.nt1 is clearly expressed in lung at low levels, but Gene561.nt2 is not (Figure 8).

5 The genomic structure of Gene 561 comprises 21 exons and spans ~200 Kb.

ATP has been shown to stimulate vagal afferent nerve terminals in the lung. This can lead to local axon and central vagal reflexes, which are known to play a major role in neurogenic inflammation and bronchoconstriction. Nocturnal asthma characterized by acute bronchoconstriction in the morning has been associated with platelet activation, which releases large amounts of ATP, and augmentation of vagal tone (Schulman et al., 1999, *Am. J. Respir. Cell Mol. Biol.* 20:530-537). It is possible that Gene 561 recruits synaptic vesicles for neurotransmitter release at the afferent nerve terminals in lung. This, in turn, may be important for bronchoconstriction/dilation. Accordingly, the Gene 561 SNPs that show association with asthma (Table 10, Figures 27A-27K, and Figures 28A-28C) may disrupt the function, stability, or expression of the encoded protein. The altered Gene 561 protein may cause an increase or decrease of neurotransmitter, resulting in augmentation of the vagal tone, and leading to bronchoconstriction. The sum of these data indicates that Gene 561 is involved in the pathophysiology of respiratory disorders including asthma.

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3. Functional Role of Gene 757 in Asthma and Related Diseases

Immunocytochemical studies have shown that both TGF- β (transforming growth factor β) and EGFR1 (epidermal growth factor receptor) are highly expressed in areas of bronchial epithelial injury, and that these parallel pathways operate to repair epithelial cells (Puddicombe et al., 2000, *FASEB J.* 14:1362-1374). EGFR1 stimulates epithelial repair, while TGF- β regulates the production of profibrogenic growth factors and proinflammatory cytokines leading to extracellular matrix synthesis. TGF- β also acts in the WNT signaling pathway, which functions in a variety

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developmental processes, including cell differentiation, cell polarity, cell migration, and cell proliferation (Calvo et al., 2000, *PNAS* 97:12776-12781). The WNT components activate the frizzled receptors, which stabilize β -catenin. This, in turn, activates the expression of target genes in the nucleus (Kühl et al., 2000, *TIG* 16:279-283).

Gene 757 is frizzled 10 (FZD10), a putative receptor for Wnt-7a (Kawakami et al., 2000, *Develop. Growth Differ.* 42:561-569). The nucleic acid sequence of Gene 757 corresponds to SEQ ID NO: 90, and the encoded amino acid sequence corresponds to SEQ ID NO: 153 (Figures 9A-9F). As determined by the experiments described herein, Gene 757 is expressed in brain, heart, skeletal muscle, colon, thymus, spleen, kidney, small intestine, placenta, and lung (Figure 6). The transcript size of Gene 757 is 3.6 Kb, of which 3253 bp have been identified (Figure 6). The transcript is contiguous with genomic DNA, indicating that Gene 757 is an intronless gene. The Gene 757 ORF is 1746 bp long and encodes a 581 amino acid protein. The 3' untranslated region is 1052 bp long, and 456 bp of the 5' UTR has been sequenced.

The FZD10 protein is a receptor composed of a seven-transmembrane repeat with an N-terminal cysteine-rich domain and a C-terminal Ser/Thr-XXX-Val motif. FZD10 shares 65.7% overall amino acid identity with FZD9 (Koike et al., 1999, *Biochem. Biophys. Res. Commun.* 262:39-43). Frizzled 10 is a cell surface receptor for the secreted glycoprotein Wnt-7a. In accordance with one model of respiratory diseases, the WNT signaling gene acts in concert with the frizzled 10 receptor to trigger a signal transduction pathway leading to the activation of genes involved in bronchial epithelial repair. Thus, Gene 757 SNPs that are associated with the asthma phenotype (Table 10 and Figures 9A-9F) may alter the signal transduction pathway, causing either the over- or underexpression of genes involved in bronchial epithelium repair. This alteration, in turn, may result in the activation of the epithelial-mesenchymal trophic unit in the lung, placing the bronchial epithelium in a "state of repair"

mode, and leading to airway remodeling (Holgate et al., 1999, *Clin. Exp. Allergy*. Suppl2:90-95). The sum of these data indicate that Gene 757 (FZD10) is directly involved in the pathophysiology of respiratory disorders including asthma.

5 **EXAMPLE 15: PROTEIN EXPRESSION AND PURIFICATION**

Expression and purification of the chromosome 12q23-qter proteins of the invention can be performed essentially as follows. Nucleotide sequences (e.g., one or more of SEQ ID NO:1 to SEQ ID NO:92 and SEQ ID NO:156 to SEQ ID NO:4684) are prepared by polymerase chain reaction
 10 (PCR). Synthetic oligonucleotide primers specific for the 5' and 3' ends of the nucleotide sequences are designed and purchased from Life Technologies (Gaithersburg, MD). All forward primers (specific for the 5' end of the sequence) are designed to include an *Nco*I cloning site at the 5' terminus. These primers are designed to permit initiation of protein
 15 translation at the methionine residue encoded within the *Nco*I site followed by a valine residue and the protein encoded by the nucleotide sequence. All reverse primers (specific for the 3' end of the sequence) include an *Eco*RI site at the 5' terminus to permit cloning of the sequence into the reading frame of the pET-28b expression vector (Novagen). The pET-28b vector
 20 provides a sequence encoding an additional 20 carboxyl-terminal amino acids including six histidine residues, which comprise the His-Tap affinity tag.

Genomic DNA prepared from the 12q23-qter including the BAC sequences including RPCI-11_0899A17, RPCI-11_0666B20, RPCI-
 25 11_0723P10, RPCI-11_0831E16, RPCI-11_0932D22 and RPCI-11_0702C13 (SEQ ID NO:719 to SEQ ID NO:978; Table 3A) and BAC end sequence (SEQ ID NO:156 to SEQ ID NO:693) region is used as the template for PCR amplification (Ausubel et al, 1994). For PCR amplification, cDNA (50 ng) is introduced into a reaction vial containing 2 mM MgCl₂, 1 μM
 30 synthetic primers (forward and reverse primers complementary to and flanking a defined 12q23-qter region), 0.2 mM of each of dNTP (dATP,

dGTP, dCTP, and dTTP), and 2.5 U heat stable DNA polymerase (Amplitaq, Roche Molecular Systems, Inc., Branchburg, NJ) in a final volume of 100 μ l.

Upon completion of thermal cycling reactions, each sample of amplified DNA is purified using the Qiaquick Spin PCR purification kit (QIAGEN, Gaithersburg, MD). PCR products are subjected to digestion with the restriction endonucleases, e.g., *Nco*I and *Eco*RI (New England BioLabs, Beverly, MA) (Ausubel et al, 1994). The digested DNA is subjected to electrophoresis on 1.0% NuSeive (FMC BioProducts, Rockland, ME) agarose gels. The gel is incubated with ethidium bromide, and the digested DNA is visualized with long-wave UV irradiation. The DNA fragments are isolated from the agarose gel, and are purified using the GeneClean Kit protocol (BIO 101, Vista, CA).

The pET-28b vector is prepared for cloning by digestion with restriction endonucleases, e.g., *Nco*I and *Eco*RI (New England BioLabs, Beverly, MA) (Ausubel et al, 1994). The digested pET-28b expression vector is ligated to the gel-isolated DNA fragments (Ausubel et al., 1994). The ligated product is used to transform *E. coli* (e.g., BL21) (Ausubel et al, 1994) as follows. Briefly, 1 μ l of ligation reaction is mixed with 50 μ l of electrocompetent BL21 cells, and the cells are subjected to a high voltage pulse. Following this, cells are incubated in 0.45 ml SOC medium (0.5% yeast extract, 2.0% tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, and 20 mM glucose) at 37°C with shaking for 1 hr. Cells are then spread on LB agar plates containing 25 μ g/ml kanamycin sulfate, and grown overnight. Transformant BL21 colonies are then isolated and analyzed to evaluate cloned inserts, as described below.

Individual BL21 transformant colonies are analyzed by PCR amplification. The PCR reaction uses the same forward and reverse primers specific for the 12q23-qter region sequences that are used in the cloning step. Successful amplification verifies the ligation of the sequence in the expression vector (Ausubel et al., 1994). Individual BL21 colonies containing pET-28b vectors with 12q23-qter region nucleotide sequences

are inoculated into 5 ml of LB broth plus 25 µg/ml kanamycin sulfate, and grown overnight. The following day, plasmid DNA is isolated and purified using the QIAGEN plasmid purification protocol (QIAGEN Inc., Chatsworth, CA).

- 5 The pET vector can be propagated in any *E. coli* K-12 strain, e.g., HMS174, HB101, JM109, DH5, and the like, for purposes of cloning or plasmid preparation. Hosts for expression include *E. coli* strains containing a chromosomal copy of the gene for T7 RNA polymerase. These hosts are lysogens of bacteriophage DE3, a lambda derivative that carries the lacI
- 10 gene, the lacUV5 promoter, and the gene for T7 RNA polymerase. T7 RNA polymerase is induced by addition of isopropyl-β-D-thiogalactoside (IPTG), and the T7 RNA polymerase transcribes any target plasmid containing a functional T7 promoter, such as pET-28b, carrying its gene of interest. Strains include, for example, BL21(DE3) (Studier et al., 1990, *Meth.*
- 15 *Enzymol.*, 185:60-89).

To express the recombinant sequence, 50 ng of plasmid DNA are isolated as described above to transform competent BL21(DE3) bacteria as described above (provided by Novagen as part of the pET expression kit). The lacZ gene (β-galactosidase) is expressed in the pET-System as

20 described for the 12q23-qter region recombinant constructions. Transformed cells are grown in SOC medium for 1 hr, and then plated on LB plates containing 25 µg/ml kanamycin sulfate. The following day, the colonies are pooled and grown in LB medium containing kanamycin sulfate (25 µg/ml) to an optical density at 600 nm of 0.5 to 1.0 OD units. At that

25 point, 1 mM IPTG is added to the culture for 3 hr to induce gene expression of the 12q23-qter sequences.

After induction of gene expression with IPTG, cells are collected by centrifugation in a Sorvall RC-3B centrifuge at 3500 x g for 15 min at 4°C. Pellets are resuspended in 50 ml of cold mM Tris-HCl, pH 8.0, 0.1 M NaCl,

30 and 0.1 mM EDTA (STE buffer). Cells are then centrifuged at 2000 x g for

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Although the invention has been set forth in detail, one skilled in the art will recognize that numerous changes and modifications can be made, and that such changes and modifications may be made without departing from the spirit and scope of the invention.